

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/82, 15/79, 15/54, A01H 5/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 97/04113</b> <b>(43) International Publication Date:</b> 6 February 1997 (06.02.97)
<b>(21) International Application Number:</b> PCT/EP96/03053 <b>(22) International Filing Date:</b> 12 July 1996 (12.07.96) <b>(30) Priority Data:</b> 9514437.4 14 July 1995 (14.07.95) GB <b>(71) Applicant (for all designated States except US):</b> DANISCO A/S [DK/DK]; Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> POULSEN, Peter [DK/DK]; Holsteinsgade 52, 3, DK-2100 Copenhagen K (DK). <b>(74) Agents:</b> HARDING, Charles, Thomas et al.; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13<sup>bis</sup> separately from the description.</i> <i>Date of receipt by the International Bureau:</i> 20 August 1996 (20.08.96)
<b>(54) Title:</b> INHIBITION OF GENE EXPRESSION  <b>(57) Abstract</b>  A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.		

**BEST AVAILABLE COPY**

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## INHIBITION OF GENE EXPRESSION

The present invention relates to a method of inhibiting gene expression, particularly inhibiting gene expression in a plant. The present invention also relates to a nucleotide sequence useful in the method. In addition, the present invention relates to a promoter that is useful for expressing the nucleotide sequence.

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylose consists essentially of straight chains of  $\alpha$ -1-4-linked glycosyl residues. Amylopectin comprises chains of  $\alpha$ -1-4-linked glycosyl residues with some  $\alpha$ -1-6 branches. The branched nature of amylopectin is accomplished by the action of *inter alia* an enzyme commonly known as the starch branching enzyme ("SBE"). SBE catalyses the formation of branch points in the amylopectin molecule by adding  $\alpha$ -1,4 glucans through  $\alpha$ -1,6-glucosidic branching linkages. The biosynthesis of amylose and amylopectin is schematically shown in Figure 1, whereas the  $\alpha$ -1-4-links and the  $\alpha$ -1-6 links are shown in Figure 2.

It is known that starch is an important raw material. Starch is widely used in the food, paper, and chemical industries. However, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties.

Within the past few years it has become desirable to make genetically modified plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In this regard, June Bourque provides a detailed summary of antisense strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149).

Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences (for example see the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3) there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity.

According to a first aspect of the present invention there is provided a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence partially or completely codes (is) an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is a sense exon sequence normally associated with the intron.

According to a second aspect of the present invention there is provided a method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

According to a third aspect of the present invention there is provided a sequence comprising the nucleotide sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof.

According to a fourth aspect of the present invention there is provided a promoter comprising the sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

According to a fifth aspect of the present invention there is provided a construct capable of comprising or expressing the present invention.

5 According to a sixth aspect of the present invention there is provided a vector comprising or expressing the present invention.

According to a seventh aspect of the present invention there is provided a cell, tissue or organ comprising or expressing the present invention.

10 According to an eighth aspect of the present invention there is provided a transgenic starch producing organism comprising or expressing the present invention. According to a ninth aspect of the present invention there is provided a starch obtained from the present invention.

15 According to a tenth aspect of the present invention there is provided pBEA11 (NCIMB 40754). According to an eleventh aspect of the present invention there is provided a sense nucleotide sequence that is obtainable from  $\lambda$ -SBE 3.2 (NCIMB 40751) or  $\lambda$ -SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.

20 A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

25 In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

30 Thus, the present invention provides a method of preparing tailor-made starches in plants which could replace the post-harvest modified starches.

Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a more beneficial effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

5

An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity. With regard to this advantage of the present invention it is to be noted that there is some

10

degree of homology between coding regions of SBEs. However, there is little or no homology with the intron sequences of SBEs. Thus, sense intron expression provides a mechanism to affect selectively the expression of a particular SBE. This advantageous aspect could be used, for example, to reduce or eliminate a particular SBE enzyme and replace that enzyme with another enzyme which can be another branching enzyme or even a recombinant version of the affected enzyme or even a hybrid enzyme which could for example comprise part of a SBE enzyme from one source and at least a part of another SBE enzyme from another source. This particular feature of the present invention is covered by the combination aspect of the present invention which is discussed in more detail later.

15

20

Thus the present invention provides a mechanism for selectively affecting SBE activity. This is in contrast to the prior art methods which are dependent on the use of for example antisense exon expression whereby it would not be possible to introduce new SBE activity without affecting that activity as well.

25

Preferably with the first aspect of the present invention starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

30

Preferably with the first or second aspect of the present invention the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

Preferably with the fourth aspect of the present invention the promoter is in combination with a gene of interest ("GOI").

Preferably the enzymatic activity is reduced or eliminated.

5

Preferably the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

10

Preferably the nucleotide sequence codes, partially or completely, for two or more introns and wherein each intron is in a sense orientation.

Preferably the nucleotide sequence comprises at least 350 nucleotides (e.g. 350 bp), more preferably at least 500 nucleotides (e.g. 500 bp).

15

Preferably the nucleotide sequence comprises the sequence shown as any one of SEQ. I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

20

Preferably the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ. I.D. No. 14 or a variant, derivative or homologue thereof.

Preferably the transgenic starch producing organism is a plant.

25

A preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

30

A more preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; and wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

The term "nucleotide" in relation to the present invention includes DNA and RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant DNA techniques.

The term "intron" is used in its normal sense as meaning a segment of nucleotides, usually DNA, that does not encode part or all of an expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides, usually DNA, encoding part or all of an expressed protein or enzyme.

Thus, the term "intron" refers to gene regions that are transcribed into RNA molecules, but which are spliced out of the RNA before the RNA is translated into a protein. In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as any one of



the sense sequences shown as SEQ.I.D. No.s 1-13. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

10 Likewise, the terms "variant" or "homologue" or "fragment" in relation to the promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective promoter sequence providing the resultant promoter sequence allows expression of a GOI, preferably wherein the resultant promoter sequence has at least the same effect as SEQ.I.D. No. 14. In particular, the term  
15 "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide sequence according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is  
20 more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

25 The intron sequence of the present invention can be any one or all of the intron sequences of the present invention, including partial sequences thereof, provided that if partial sense sequences are used (i.e. sequences that are not or do not comprise any one or more of the full sequences shown as SEQ.I.D. No.1-13) the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences  
30 that are shorter than any one of the full sense sequences shown as SEQ.I.D.No.s 1 to 13 but which comprise nucleotides that are adjacent the respective exon or exons.

With regard to the second aspect of the present invention (i.e. specifically affecting SBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of the SBE gene (but not sense exon sequences naturally associated with the intron sequence), including complete or partial sequences thereof, providing the nucleotide sequences can affect SBE activity, preferably wherein the nucleotide sequences reduce or eliminate SBE activity. Preferably, the nucleotide sequence of the second aspect of the present invention does not comprise sense exon sequences.

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E. Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the sense nucleotide sequence aspect of the present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. The terms do not cover the natural combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence of the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Shl*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. In one aspect, preferably the promoter is the patatin promoter or the E35S promoter. In another aspect, preferably the promoter is the SBE promoter.

If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. By way of example, the promoter for the nucleotide sequence of the present invention can be the  $\alpha$ -Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the  $\alpha$ -Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. Alternatively, the promoter for the nucleotide sequence of the present invention can be the  $\alpha$ -Amy 3 promoter (otherwise known as the Amy 3

promoter, the Amy 351 promoter or the  $\alpha$ -Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994.

5 The present invention also encompasses the use of a promoter to express a nucleotide sequence according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous. In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses the nucleotide sequence of the present  
10 invention in a more specific manner such as in just one specific tissue type or organ. The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a gene coding for the enzyme of the present invention in at  
15 least one (but not all) specific tissue of the original promoter. Examples of partial inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof.  
20 Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part. Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

25

The construct and/or the vector of the present invention may include a transcriptional termination region.

30 The nucleotide according to the present invention can be expressed in combination (but not necessarily at the same time) with an additional construct. Thus the present invention also provides a combination of constructs comprising a first construct comprising the nucleotide sequence according to the present invention operatively

linked to a first promoter; and a second construct comprising a GOI operatively linked to a second promoter (which need not be the same as the first promoter). With this aspect of the present invention the combination of constructs may be present in the same vector, plasmid, cells, tissue, organ or organism. This aspect of the present invention also covers methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a plant. With this aspect of the present invention the second construct does not cover the natural combination of the gene coding for an enzyme ordinarily associated with the wild type gene promoter when they are both in their natural environment.

An example of a suitable combination would be a first construct comprising the nucleotide sequence of the present invention and a promoter, such as the promoter of the present invention, and a second construct comprising a promoter, such as the promoter of the present invention, and a GOI wherein the GOI codes for another starch branching enzyme either in sense or antisense orientation.

The above comments relating to the term "construct" for the sense nucleotide aspect of the present invention are equally applicable to the term "construct" for the promoter aspect of the present invention. In this regard, the term includes the promoter according to the present invention directly or indirectly attached to a GOI.

The term "GOI" with reference to the promoter aspect of the present invention or the combination aspect of the present invention means any gene of interest, which need not necessarily code for a protein or an enzyme - as is explained later. A GOI can be any nucleotide sequence that is either foreign or natural to the organism in question, for example a plant.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. An example of such a GOI is the nucleotide sequence according to the present invention.

5 The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code  
10 for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and  $\alpha$ -galactosidase. The GOI  
15 can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for  $\alpha$ -amylase, a protease or a glucanase. Alternatively, the GOI can be a nucleotide sequence according to the present invention.

The GOI can be the nucleotide sequence coding for the arabinofuranosidase enzyme  
20 which is the subject of our co-pending UK patent application 9505479.7. The GOI can be the nucleotide sequence coding for the glucanase enzyme which is the subject of our co-pending UK patent application 9505475.5. The GOI can be the nucleotide sequence coding for the  $\alpha$ -amylase enzyme which is the subject of our co-pending UK patent application 9413439.2. The GOI can be the nucleotide sequence coding for  
25 the  $\alpha$ -amylase enzyme which is the subject of our co-pending UK patent application 9421290.9. The GOI can be any of the nucleotide sequences coding for the  $\alpha$ -glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397.

30 In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase.

Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

5

As mentioned above, the present invention provides a mechanism for selectively affecting a particular enzymatic activity.

10

In an important application of the present invention it is now possible to reduce or eliminate expression of a genomic nucleotide sequence coding for a genomic protein or enzyme by expressing a sense intron construct for that particular genomic protein or enzyme and (e.g. at the same time) expressing a recombinant version of that enzyme or protein - in other words the GOI is a recombinant nucleotide sequence coding for the genomic enzyme or protein. This application allows expression of desired recombinant enzymes and proteins in the absence of (or reduced levels of) respective genomic enzymes and proteins. Thus the desired recombinant enzymes and proteins can be easily separated and purified from the host organism. This particular aspect of the present invention is very advantageous over the prior art methods which, for example, rely on the use of anti-sense exon expression which methods also affect expression of the recombinant enzyme.

15

20

25

30

Thus, a further aspect of the present invention relates to a method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron. Additional aspects cover the combination of those nucleotide sequences including their incorporation in constructs, vectors, cells, tissues and transgenic organisms.

Therefore the present invention also relates to a combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding the enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

The GOI may even code for one or more introns but in an antisense orientation, such as any one or more of the antisense intron sequences presented in the attached sequence listings. For example, the present invention also covers the expression of for example a sense intron (e.g. SEQ.I.D.No. 1) in combination with for example an antisense sense intron which preferably is not complementary to the sense intron sequence (e.g. SEQ.I.D.No. 16).

The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cereals such as wheat, maize, and barley; fruit; trees; flowers; and other plant crops. Preferably, the term means "potato".



The term "transgenic organism" in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products obtained therefrom; and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide sequence of the present invention is incorporated in the genome of the organism. Preferably the transgenic organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Sambrook *et al.* in Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

Even though the enzyme according to the present invention and the nucleotide sequence coding for same are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is

capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant.

5 The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.* (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

10 One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.* (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. Several different Ti and Ri plasmids have been constructed which are suitable for the  
15 construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

20 The nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appears to be essential for insertion of modified T-DNA into the plant genome.

25 As will be understood from the above explanation, if the organism is a plant the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

30 Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof. As these plasmids are well-known and widely employed in the construction of transgenic

plants; many vector systems exist which are based on these plasmids or derivatives thereof.

5 In the construction of a transgenic plant the nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the nucleotide sequence or construct of the present invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the present invention, which DNA is subsequently transferred into the plant cell to be modified.

15 If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System-Offset-drukkerij Kanters B.B., Alblasterdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An *et al.*, EMBO J. (1985) 4:277-284.

25 Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be performed in or on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the GOI (such as the nucleotide sequence according to the present invention) and, optionally, a promoter, a plant to be infected is wounded, e.g. by cutting the plant with a razor blade or puncturing the plant with a needle or rubbing the plant with an abrasive.

5 The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

15 Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

20 Further teachings on plant transformation may be found in EP-A-0449375.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In this way, the nucleotide or construct of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is then used for the transformation in *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same

25

30

or different plasmid.

After the introduction of the nucleotide sequence or construct according to the present invention in the plants the presence and/or insertion of further DNA sequences may  
5 be necessary - such as to create combination systems as outlined above (e.g. an organism comprising a combination of constructs).

The above commentary for the transformation of prokaryotic organisms and plants with the nucleotide sequence of the present invention is equally applicable for the  
10 transformation of those organisms with the promoter of the present invention.

In summation, the present invention relates to affecting enzyme activity by expressing sense intron sequences.

15 Also, the present invention relates to a promoter useful for the expression of those sense intron sequences.

The following samples have been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine  
20 Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 13 July 1995:

NCIMB 40754 (which refers to pBEA 11 as described herein);

25 NCIMB 40751 (which refers to  $\lambda$ -SBE 3.2 as described herein), and

NCIMB 40752 (which refers to  $\lambda$ -SBE 3.4 as described herein).

A highly preferred embodiment of the present invention therefore relates to a method  
30 of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely,

for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754 or a variant, derivative or homologue thereof.

A more highly preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof; and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754, or a variant, derivative or homologue thereof.

The present invention will now be described only by way of example, in which reference is made to the following attached Figures:

Figure 1, which is a schematic representation of the biosynthesis of amylose and amylopectin;

Figure 2, which is a diagrammatic representation of the  $\alpha$ -1-4-links and the  $\alpha$ -1-6 links of amylopectin;

Figure 3, which is a diagrammatic representation of the exon-intron structure of a genomic SBE clone;

Figure 4, which is a plasmid map of pPATA1, which is 3936 bp in size;

Figure 5, which is a plasmid map of pABE7, which is 5106 bp in size;

5 Figure 6, which is a plasmid map of pVictorIV Man, which is 7080 bp in size;

Figure 7, which is a plasmid map of pBEA11, which is 9.54 kb in size;

10 Figure 8, which shows the full genomic nucleotide sequence for SBE including the promoter, exons and introns;

Figure 9, which is a plasmid map of pVictor5a, which is 9.12 kb in size; and

15 Figure 10, which is a plasmid map of pBEP2, which is 10.32 kb in size.

Figures 1 and 2 were referred to above in the introductory description concerning starch in general. As mentioned, Figure 3 is a diagrammatic representation of the exon-intron structure of a genomic SBE clone, the sequence of which is shown in Figure 8. This clone, which has about 11.5 k base pairs, comprises 14 exons and 13  
20 introns. The introns are numbered in increasing order from the 5' end to the 3' end and correspond to SEQ.I.D.No.s 1-13, respectively. Their respective antisense intron sequences are shown as SEQ.I.D.No.s 15-27.

In more detail, Figures 3 and 8 present information on the 11468 base pairs of a  
25 potato SBE gene. The 5' region from nucleotides 1 to 2082 contain the promoter region of the SBE gene. A TATA box candidate at nucleotide 2048 to 2051 is boxed. The homology between a potato SBE cDNA clone (Poulsen & Kreiberg (1993) Plant Physiol 102: 1053-1054) and the exon DNAs begin at 2083 bp and end at 9666 bp. The homology between the cDNA and the exon DNA is indicated by nucleotides in  
30 upper case letters, while the translated amino acid sequences are shown in the single letter code below the exon DNA. Intron sequences are indicated by lower case letters.

Figure 7 is a plasmid map of pBEA7, which is 9.54 k base pairs in size. Plasmid pBEA 11 comprises the first intron sequence of the potato SBE gene. This first intron sequence, which has 1177 base pairs, is shown in Figure 3 and lies between the first exon and the second exon.

5

These experiments and aspects of the present invention are now discussed in more detail.

### EXPERIMENTAL PROTOCOL

10

#### ISOLATION, SUBCLONING IN PLASMIDS, AND SEQUENCING OF GENOMIC SBE CLONES

15

Various clones containing the potato SBE gene were isolated from a Desiree potato genomic library (Clontech Laboratories Inc., Palo Alto CA, USA) using radioactively labelled potato SBE cDNA (Poulsen & Kreiberg (1993) Plant Physiol. 102:1053-1054) as probe. The fragments of the isolated  $\lambda$ -phages containing SBE DNA ( $\lambda$ SBE 3.2 - NCIMB 40751 - and  $\lambda$ SBE-3.4 - NCIMB 40752) were identified by Southern analysis and then subcloned into pBluescript II vectors (Clontech Laboratories Inc., Palo Alto CA, USA).  $\lambda$ SBE 3.2 contains a 15 kb potato DNA insert and  $\lambda$ SBE-3.4 contains a 13 kb potato DNA insert. The resultant plasmids were called pGB3, pGB11, pGB15, pGB16 and pGB25 (see discussion below). The respective inserts were then sequenced using the Pharmacia Autoread Sequencing Kit (Pharmacia, Uppsala) and a A.L.F. DNA sequencer (Pharmacia, Uppsala).

25

In total, a stretch of 11.5 kb of the SBE gene was sequenced. The sequence was deduced from the above-mentioned plasmids, wherein: pGB25 contains the sequences from 1 bp to 836 bp, pGB15 contains the sequences from 735 bp to 2580 bp, pGB16 contains the sequences from 2580 bp to 5093 bp, pGB11 contains the sequences from 3348 bp to 7975 bp, and pGB3 contains the sequences from 7533 bp to 11468 bp.

30



In more detail, pGB3 was constructed by insertion of a 4 kb *EcoRI* fragment isolated from  $\lambda$ SBE 3.2 into the *EcoRI* site of pBluescript II SK (+). pGB11 was constructed by insertion of a 4.7 kb *XhoI* fragment isolated from  $\lambda$ SBE 3.4 into the *XhoI* site of pBluescript II SK (+). pGB15 was constructed by insertion of a 1.7 kb *SpeI* fragment isolated from  $\lambda$ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). pGB16 was constructed by insertion of a 2.5 kb *SpeI* fragment isolated from  $\lambda$ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). For the construction of pGB25 a PCR fragment was produced with the primers

5' GGA ATT CCA GTC GCA GTC TAC ATT AC 3'

and

5' CGG GAT CCA GAG GCA TTA AGA TTT CTG G 3'

and  $\lambda$ SBE 3.4 as a template.

The PCR fragment was digested with *BamHI* and *EcoRI*, and inserted in pBluescript II SK (+) digested with the same restriction enzymes.

#### CONSTRUCTION OF PLASMID pBEA11

The SBE intron 1 was amplified by PCR using the oligonucleotides

5' CGG GAT CCA AAG AAA TTC TCG AGG TTA CAT GG 3'

and

5' CGG GAT CCG GGG TAA TTT TTA CTA ATT TCA TG 3'

and the  $\lambda$ SBE 3.4 phage containing the SBE gene as template.

The PCR product was digested with *Bam*HI and inserted in a sense orientation in the *Bam*HI site of plasmid pPATA1 (described in WO 94/24292) between the patatin promoter and the 35S terminator. This construction, pABE7, was digested with *Kpn*I, and the 2.4 kb "patatin promoter-SBE intron 1- 35S terminator" *Kpn*I fragment was isolated and inserted in the *Kpn*I site of the plant transformation vector pVictorIV Man yielding plasmid pBEA11.

## PRODUCTION OF TRANSGENIC POTATO PLANTS

### Axenic stock cultures

Shoot cultures of *Solanum tuberosum* 'Bintje' and 'Dianella' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), *Physiol. Plant.* 18: 100-127, in addition containing 2  $\mu$ M silver thiosulphate at 25°C and 16 h light/8 h dark.

The cultures were subcultured after approximately 40 days. Leaves were then cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

### Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) were cut into internodal segments (approximately 0.8 cm). The segments were placed into liquid LS-substrate containing the transformed *Agrobacterium tumefaciens* containing the binary vector of interest. The *Agrobacterium* were grown overnight in YMB-substrate (di-potassium hydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the *Agrobacterium* strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments were left in the suspension of *Agrobacterium* for 30 minutes and then the excess of bacteria were removed by blotting the segments on sterile filter paper.

#### Co-cultivation

The shoot segments were co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants were covered with sterile filter papers, and the petri dishes were placed at 25°C and 16 h light/ 8 dark.

#### "Washing" procedure

After the 48 h on the co-cultivation substrate the segments were transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers were gently shaken and by this procedure the major part of the *Agrobacterium* was either washed off the segments and/or killed.

#### Selection

After the washing procedure the segments were transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used. The segments were sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continued for 3-4 months.

#### Rooting of regenerated shoots

The regenerated shoots were transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

The transgenic genotype of the regenerated shoot were verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154). Plants which were not positive in any of these assays were discarded or used as controls. Alternatively, the transgenic plants could be verified by performing a GUS assay on the co-introduced  $\beta$ -glucuronidase gene according to Hodal, L. *et al*. (Pl. Sci. (1992), 87: 115-122).

#### 10     Transfer to soil

The newly rooted plants (height approx. 2-3 cms) were transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m<sup>2</sup>/sec). When the plants were well established they were transferred to the greenhouse, where they were grown until tubers had developed and the upper part of the plants were senescing.

#### Harvesting

20     The potatoes were harvested after about 3 months and then analysed.

#### BRANCHING ENZYME ANALYSIS

25     The SBE expression in the transgenic potato lines were measured using the SBE assays described by Blennow and Johansson (Phytochemistry (1991) 30:437-444) and by standard Western procedures using antibodies directed against potato SBE.

#### STARCH ANALYSIS

30     Starch was isolated from potato tubers and analysed for the amylose:amylopectin ratio (Hovenkamp-Hermelink et al. (1988) Potato Research 31:241-246). In addition, the chain length distribution of amylopectin was determined by analysis of isoamylase

digested starch on a Dionex HPAEC. The number of reducing ends in isoamylase digested starch was determined by the method described by N. Nelson (1944) J. Biol.Chem. 153:375-380.

5 The results revealed that there was a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch SBE in the transgenic plants.

#### CONSTRUCTION OF SBE PROMOTER CONSTRUCT

10

An SBE promoter fragment was amplified from  $\lambda$ -SBE 3.4 using primers:

5' CCA TCG ATA CTT TAA GTG ATT TGA TGG C 3'

15

and

5' CGG GAT CCT GTT CTG ATT CTT GAT TTC C 3'.

20

The PCR product was digested with *Cla*I and *Bam*HI. The resultant 1.2 kb fragment was then inserted in pVictor5a (see Figure 9) linearised with *Cla*I and *Bg*III yielding pBEP2 (see Figure 10).

#### STARCH BRANCHING ENZYME MEASUREMENTS OF POTATO TUBERS

25

Potatoes from potato plants transformed with pBEA11 were cut in small pieces and homogenised in extraction buffer (50 mM Tris-HCl pH 7.5, Sodium-dithionit (0.1 g/l), and 2 mM DTT) using a Ultra-Turax homogenizer; 1 g of Dowex xl. was added pr. 10 g of tuber. The crude homogenate was filtered through a miracloth filter and centrifuged at 4°C for 10 minutes at 24.700 g. The supernatant was used for starch branching enzyme assays.

30

The starch branching enzyme assays were carried out at 25 °C in a volume of 400  $\mu$ l composed of 0.1 M Na citrate buffer pH 7.0, 0.75 mg/ml amylose, 5 mg/ml bovine serum albumin and the potato extract. At 0, 15 30 and 60 minutes aliquots of 50  $\mu$ l were removed from the reaction into 20  $\mu$ l 3 N HCl. 1 ml of iodine solution was added and the decrease in absorbance at 620 nm was measured with an ELISA spectrophotometer.

The starch branching enzyme (SBE) levels in tuber extracts were measured from 24 transgenic Dianella potato plants transformed with plasmid pBEA11.

The results showed that the BEA11 transgenic lines produced tubers which have SBE levels that are only 10 % to 15 % of the SBE levels found in non transformed Dianella plants.

## SUMMATION

The above-mentioned examples relate to the isolation and sequencing of a gene for potato SBE. The examples further demonstrate that it is possible to prepare SBE intron constructs. These SBE intron constructs can be introduced into plants, such as potato plants. After introduction, a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch in plants can be achieved.

Without wishing to be bound by theory it is believed that the expressed sense intron nucleotide sequence according to the present invention affects enzymatic activity *via* co-suppression and/or trans-activation. Reviews of these mechanisms has been published by Finnegan and McElroy (1994 Biotechnology 12 pp 883 - 887) and Matzke and Matzke (1995 TIG 11 No. 1 pp 1 - 3). By these mechanisms, it is believed that the sense introns of the present invention reduce the level of plant enzyme activity (in particular SBE activity), which in turn for SBE activity is believed to influence the amylose:amylopectin ratio and thus the branching pattern of amylopectin.

Thus, the present invention provides a method wherein it is possible to manipulate the starch composition in plants, or tissues or cells thereof, such as potato tubers, by reducing the level of SBE activity by using sense intron sequences.

5 In summation the present invention therefore relates to the surprising use of sense intron sequences in a method to affect enzymatic activity in plants.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention. For example, it may  
10 be possible to use antisense promoter sequences to affect enzymatic activity, such as antisense SBE promoter - such as a nucleotide sequence comprising the nucleotide sequence shown as SEQ. I.D. No. 28 or a variant, derivative or homologue thereof.

The following pages present a number of sequence listings which have been  
15 consecutively numbered from SEQ.I.D. No. 1 - SEQ.I.D. No. 29. In brief, SEQ.I.D. No. 1 - SEQ.I.D. No. 13 represent sense intron sequences (genomic DNA); SEQ.I.D. No. 14 represents the SBE promoter sequence (genomic sequence); SEQ.I.D. No. 15 - SEQ.I.D. No. 27 represent antisense intron sequences; and SEQ.  
I.D. No. 28 represents the sequence complementary to the SBE promoter sequence -  
20 i.e. the SBE promoter sequence in antisense orientation. The full genomic nucleotide sequence for SBE including the promoter, exons and introns is shown as SEQ. I.D. No. 29 (see Figures 3 and 8 which highlight particular gene features).

## SEQUENCE INFORMATION

SEQ.I.D. No. 1

Intron 1 sequence (1167 bp).

GTAATTTTACTAATTTTCATGTTAATTTCAATTATTTTTCAGCCTTTCATTTCATTTTCCAATATATCT  
GGATCATCTCCTTAGTTTTTTTATTTTATTTTATAATATCAAATATGGAAGAAAAATGACACTTGTAG  
AGCCATATGTAAGTATCATGTGACAAATTTGCAAGGTGGTTGAGTGTATAAAATTCAAAAATTGAGAGA  
TGGAGGGGGGGTGGGGGBARAGACAATATTTAGAAAGAGTGTCTAGGAGGTTATGGAGGACACGGATG  
AGGGGTAGAAGGTTAGTTAGGTATTTGAGTGTGTCTGGCTTATCCTTTTCATACTAGTAGTCGTGGAAT  
TATTTGGGTAGTTTCTTGTTTTGTTATTTGATCTTTGTTATTCTATTTTCTGTTTCTTGACTTCGATT  
ATTGTATTATATATCTTGTCTAGTTATTGTTCTCGGTAAGAATGCTCTAGCATGCTTCCTTTAGTGT  
TTTATCATGCCTTCTTTATATTTCGCGTTGCTTTGAAATGCTTTTACTTTAGCCGAGGGTCTATTAGAAA  
CAATCTCTCTATCTCGTAAGGTAGGGGTAAAGTCCTCACCACACTCCACTTGTGGGATTACATTGTGTT  
TGTTGTTGTAAATCAATTATGTATACATAATAAGTGGATTTTTTACAACACAAATACATGGTCAAGGGC  
AAAGTTCGTAACACATAAAGGGTTCATTATATGTCCAGGGATATGATAAAAATTGTTTCTTTGTGAAAG  
TTATATAAGATTTGTTATGGCTTTTGCTGGAAACATAATAAGTTATAATGCTGAGATAGCTACTGAAGT  
TTGTTTTTTCTAGCCTTTTAAATGTACCAATAATAGATTCCGTATCGAACGAGTATGTTTTGATTACCT  
GGTCATGATGTTTCTATTTTTTACATTTTTTGGTGTGAACTGCAATTGAAAATGTTGTATCCTATGA  
GACGGATAGTTGAGAATGTGTTCTTTGTATGGACCTTGAGAAGCTCAAACGCTACTCCAATAATTTCTA  
TGAATTCAAATTCAGTTTATGGCTACCAGTCAGTCCAGAAATTAGGATATGCTGCATATACTTGTTCAA  
TTATACTGTAAATTTCTTAAGTTCTCAAGATATCCATGTAACCTCGAGAATTTCTTTGACAG

SEQ.I.D. No. 2

Intron 2 sequence (321 bp).

GTATGTTTGATAATTTATATGGTTGCATGGATAGTATATAAATAGTTGGAAAACCTTCTGGACTGGTGCT  
CATGGCATATTTGATCTGTGCACCGTGTGGAGATGTCAAACATGTGTTACTTCGTTCCGCCAATTTATA  
ATACCTTAACTTGGGAAAGACAGCTCTTTACTCCTGTGGGCATTTGTTATTTGAATTACAATCTTTATG  
AGCATGGTGTTTTACATTATCAACTTCTTTCATGTGGTATATAACAGTTTTTTAGCTCCGTTAATACCT  
TTCTTCTTTTGTATATAAACTAACTGTGGTGCATTGCTTGCBBKK

SEQ.I.D. No. 3

Intron 3 sequence (504 bp).

GTAACAGCCAAAAGTTGTGCTTTAGGCAGTTTGACCTTATTTTGAAGATGAATTGTTTATACCTACTT  
TGACTTTGCTAGAGAATTTTGCATACCGGGGAGTAAGTAGTGGCTCCATTAGGTGGCACCTGGCCATT  
TTTTTGATCTTTTAAAAAGCTGTTTGATTGGGTCTTCAAAAAAGTAGACAAGGTTTTTGGAGAAGTGAC  
ACACCCCGGAGTGTCAAGTGGCAAAGCAAAGATTTTCACTAAGGAGATTCAAAATATAAAAAAAGTATA  
GACATAAGAAGCTGAGGGGATTCAACATGTACTATACAAGCATCAATATAGTCTTAAAGCAATTTTG  
TAGAAATAAAGAAAGTCTTCTTCTGTTGCTTCACAATTTCTTCTATTATCATGAGTACTCTTTCTG  
TTCGAAATAGCTTCTTAATATTAAATTCATGATACTTTTGTGAGATTTAGCAGTTTTTTCTTGTGTA  
AACTGCTCTCTTTTTTTGACAG



## SEQ.I.D. No. 4

Intron 4 sequence (146 bp).

GTAGGTCCTGGTCTACTACAAAATAGTAGTTTGCATCATCATAACAGATTTTCCTATTAAAGCATGATG  
TTGCAGCATCATTGGCTTCTTACATGTTCTAATTGCTATTAAGGTTATGCTTCTAATTAACATCATCCA  
CAATGCAG

## SEQ.I.D. No. 5

Intron 5 sequence (218 bp).

GTTTTGTTATTTCATACCTTGAAGCTGAATTTTGAACACCATCATCACAGGCATTTTCGATTCATGTTCTT  
ACTAGTCTTGTTATGTAAGACATTTTGAAATGCAAAGTTAAAATAATTGTGTCTTTACTAATTTGGAC  
TTGATCCCATACTCTTCCCTTAACAAAATGAGTCAATTCTATAAGTGCTTGAGAACTTACTACTTCAG  
CAATTAAACAG

## SEQ.I.D. No. 6

Intron 6 sequence (198 bp).

GTATTTTAAATTTATTTCTACAATAAATTCTCAGAACAATTGTTAGATAGAATCCAAATATATAC  
GTCCTGAAAGTATAAAAGTACTTATTTTCGCCATGGGCCTTCAGAATATTGGTAGCCGCTGAATATCAT  
GATAAGTTATTTATCCAGTGACATTTTTATGTTCACTCCTATTATGTCTGCTGGATACAG

## SEQ.I.D. No. 7

Intron 7 sequence (208bp)

GTTTGTCTGTTTCTATTGCATTTTAAGGTTTCATATAGGTTAGCCACGGAAAATCTCACTCTTTGTGAGG  
TAACCAGGGTTCTGATGGATTATTCAATTTTCTCGTTTATCATTTGTTTATTCTTTTCATGCATTGTGT  
TTCTTTTCAATATCCCTCTTATTTGGAGGTAATTTTCTCATCTATTCACTTTTAGCTTCTAACCACAG

## SEQ.I.D. No. 8

Intron 8 sequence (293 bp).

GTATGTCTTACATCTTTAGATATTTTGTGATAATTACAATTAGTTTGGCTTACTTGAACAAGATTCATT  
CCTCAAATGACCTGAAGTGTGAACATCAAAGGGGTTGAACATAGAGGAAAACAACATGATGAATGT  
TTCCATTGTCTAGGGATTTCTATTATGTTGCTGAGAACAATGTCATCTTAAAAAAACATTGTTTACT  
TTTTTGTAGTATAGAAGATTACTGTATAGAGTTTGCAAGTGTGTCTGTTTGGAGTAATTGTGAAATGT  
TTGATGAAGTTGTACAG

## SEQ.I.D. No. 9

Intron 9 sequence (376 bp).

GTTCAAGTATTTTGAATCGCAGCTTGTTAAATAATCTAGTAATTTTATAGATTGCTTACTTGAAGTCTA  
CTTGGTTCTGGGGATGATAGCTCATTTTCATCTTGTCTACTTATTTTCCAACCGAATTTCTGATTTTGT  
TTTCGAGATCCAAGTATTAGATTCATTTACACTTATTACCGCCTCATTTCTACCACTAAGGCCTTGATG  
AGCAGCTTAAGTTGATTCTTTGAAGCTATAGTTTCAGGCTACCAATCCACAGCCTGCTATATTTGTTGG

ATACTTACCTTTTCTTTACAATGAAGTGATACTAATTGAAATGGTCTAAATCTGATATCTATATTTCTC  
CGTCTTTCTCCCTCATGATGAAATGCAG

SEQ.I.D. No. 10

Intron 10 sequence (172 bp).

GTAAATCATCTAAAGTTGAAAGTGTGGGTTTATGAAGTGCTTTAATTCTATCCAAGGACAAGTAGAA  
ACCTTTTACCTTCCATTCTTGATGATGGATTTTATATTATTTAATCCAATAGCTGGTCAAATTCGGT  
AATAGCTGTACTGATTAGTTACTTCACTTTGCAG

SEQ.I.D. No. 11

Intron 11 sequence (145 bp).

GTATATATGTTTTACTTATCCATGAAATTATTGCTCTGCTTGTGTTTTAATGTACTGAACAAGTTTTATG  
GAGAAGTAACTGAAACAAATCATTTTCACATTGTCTAATTTAACTCTTTTTCTGATCCTCGCATGACG  
AAAACAG

SEQ.I.D. No. 12

Intron 12 sequence (242 bp).

GTAAGGATTTGCTTGAATAACTTTTGATAATAAGATAACAGATGTAGGGTACAGTTCTCTCACCAGAAA  
GAACTGTAATTGTCTCATCCATCTTTAGTTGTATAAGATATCCGACTGTCTGAGTTCGGAAGTGTGGA  
GCCTCCTGCCCTCCCCCTGCGTTGTTTAGCTAATTTCAAAAAGGAGAAAAGTGTATTGATGATCTTTG  
TCTTCATGCTGACATACAATCTGTTCTCATGACAG

SEQ.I.D. No. 13

Intron 13 sequence (797 bp).

GTACAGTTCTTGCCGTGTGACCTCCCTTTTTATTGTGGTTTTGTTTCATAGTTATTTGAATGCGATAGAA  
GTAACTATTGATTACCGCCACAATCGCCAGTTAAGTCCTCTGAACTACTAATTTGAAAGGTAGGAATA  
GCCGTAATAAGGTCTACTTTTGGCATCTTACTGTTACAAAACAAAAGGATGCCAAAAAATTCTTCTCT  
ATCCTCTTTTTCCCTAAACCAGTGCATGTAGCTTGCACCTGCATAAACTTAGGTAAATGATCAAAAATG  
AAGTTGATGGGAACCTTAAACCGCCCTGAAGTAAAGCTAGGAATAGTCATATAATGTCCACCTTTGGTG  
TCTGCGCTAACATCAACAACAACATACCTCGTGTAGTCCACAAAGTGGTTTCAGGGGGAGGGTAGAGT  
GTATGCAAACTTACTCCTATCTCAGAGGTAGAGAGGATTTTTCAATAGACCCTTGGCTCAAGAAAAA  
AAGTCCAAAAAGAAGTAACAGAAGTGAAAGCAACATGTGTAGCTAAAGCGACCCAACTTGTGTTGGGACT  
GAAGTAGTTGTTGTTGTTGAAACAGTGCATGTAGATGAACACATGTCAGAAAATGGACAACACAGTTAT  
TTTGTGCAAGTCAAAAAAATGTACTACTATTCTTTGTGCAGCTTTATGTATAGAAAAGTTAAATAACT  
AATGAATTTTGCTAGCAGAAAAATAGCTTGAGAGAAAATTTTTATATTGAACTAAGCTAACTATATTC  
ATCTTTCTTTTGCTTCTTCTCCTTGTGTTGTGAAG

SEQ.I.D. No. 14

DNA sequence of the SBE gene promoter region.

ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTTCAGATTCTTTCGAGTTCTCAT	60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAAGTGTGCATCTGCTTCTTAGAACT	120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTCAAACTCTT	180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTCCTCAATGATGTTTACAGTGTGTG	240
TTGTCATCTGTACTTTTGCCTATTACTTGTTCCTGAGTTACATGTTAAAAAAGTGTATT	300
TTGCCATATTTGTTCTCTTATTATTATTATCATAACATTATTACAAGGAAAAGACA	360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA	420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG	480
CATAAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC	540
AACGTTAATTTAGTAATTTTGTACGTACGTATATGAAATATTGAATTAATCACATGAA	600
CGGTGGATATTATATTATGAGTTGGEATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT	660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAATAAATCTCATTTCAAGT	720
GGGATTAGAACTAGTTATTAATAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT	780
AAAGTTTTTCATTTTCATGCTAAATTTGTTAATTATTGTAATGTAGACTGCGACTGGAATT	840
ATTATAGTGTAATTTATGCATTCAGTGTAATAAATTAAGTATTGAACCTGTCTGTTTTAG	900
AAAATACTTTTATACTTTAATATAGGATTTTGTTCATGCGAATTTAAATTAATCGATATTGA	960
ACACGGAATACCAAAATTAATAAGGATACACATGGCCTTCATATGAACCGTGAACTTTTG	1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAGAATAAACTGACAAATTAATTTCTTTT	1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT	1140
GAATGATATTTCATTTTCATCCATAAGTTCAATTTGATTGTCATACCACCCATGATGTT	1200
CTGAAAAATGCTTGGCCATTACAAAGTTTATCTTAGTTCTATGAACCTTTATAAGAAGC	1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCAATAGACAAGTGTA	1320
TTAATATTGTAACCTTTGTAATTGAGTGTGTCTACATCTTATTCAATCATTAAAGGTCATT	1380
AAAATAAATTATTTTTTGACATTCTAAAACCTTTAAGCAGAATAAATAGTTTATCAATTAT	1440
TAAAAACAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT	1500
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTTT	1560
TTTAGCCTAACCAACGAATATTTGTAACTCACAACCTTGATTAAAAGGGATTACAACAA	1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAATTTGGAGGTCAAATTT	1680
TACCATAATCATTGTATTTATAATTAATTTTAAATATCTTATTTATACATATCTAGTA	1740
AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTTCATATTAGGTCAATA	1800
AATCCTTAACATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA	1860
CATGTATTATGTATACAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA	1920
AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAATATGTTTTACTTCAATTTTCG	1980
AAACTAATGGGGTCTGAGTGAAATATTTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT	2040
GTTTTTTTATAAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA	2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTTATCAAA	2160
ACCCATTTCG	

SEQ.I.D. No. 15

Intron 1 antisense sequence (1167 bp).

CTGTCAAAGAAATTCTCGAGGTTACATGGATATCTTGAGAACTTAAGAAATTTTACAGTATAATTGAAC  
AAGTATATGCAGCATATCCTAATTTCTGGACTGACTGGTAGCCATAAACTGAATTTGAATTCATAGAAA  
TTATTGGAGTAGCGTTTGAGCTTCTCAAGGTCCATACAAAGAACACATTCTCAACTATCCGTCTCATAG  
GATACAACATTTTCAATTGCAGTTCAACACCAAAAAATGTAAAAATAGAAACATCATGACCAGGTAA  
TCAAAACATACTCGTTTCGATACGGAATCTATTATTGGTACATTTAAAAGGCTAGAAAAACAACTTCA  
GTAGCTATCTCAGCATTATAACTTATTATGTTTCCAGCAAAAGCCATAACAAATCTTATATACTTTCA  
CAAAGAAACAATTTTTATCATATCCCTGGACATATAATGAACCTTTATGTGTTTCAGAACTTTGCCCTT  
GACCATGTATTTGTGTTGTAAAAATCCACTTATTATGTATACATAATTGATTTACAACAACAAACACA  
ATGTAATCCCACAAGTGGAGTGTGGTGAGGACTTTACCCCTACCTTACGAGATAGAGAGATTGTTTCTA  
ATAGACCCTCGGCTAAAGTAAAGCATTTCAAAGCAACGCGAATATAAAGAAGGCATGATAAAACACTA  
AAGGAAGCATGCTAGAGCATTCTTACCGAGGAACAATACTACGACAAGATATATAATACAATAATCGA  
AGTACAAGAAACAGAAAATAGAATAACAAAGATCAAATAACAAAACAAGAACTACCCAAATAATTCCA  
CGACTACTAGTATGAAAGGATAAGCCAGACAACACTCAAATACCTAACTAACCTTCTACCCCTCATCCG  
TGTCCTCCATAACCTCCTAGAACACTCTTTCTAAATATTGTCTYTVCCCCACCCCCCTCCATCTCTC  
AATTTTTGAATTTTATACACTCAACCACCTTGCAATTTGTCACATGATACTTACATATGGCTCTACAA  
GTGTCATTTTTCTTCCATATTTGATATTATAAAAAATAAAAAACTAAGGAGATGATCCAGATAT  
ATTGGAATGAAATGCAAAGGCTAAAAATAATTGAAATTAACATGAAATTAGTAAAAATTAC

SEQ.I.D. No. 16

Intron 2 antisense sequence (321 bp).

MMMVGCAAGCAATGCACCACAGTTAGTTTATATCAAAAAAGAAGAAAGGTATTACGGAGCTAAAACTG  
TTATATACCACATGAAAGAAGTTGATAATGTGAAAACACCATGCTCATAAGATTGTAATTCAAATAAC  
AAATGCCCCACAGGAGTAAAGAGCTGTCTTTCCCAAGTTAAGGTATTATAAATTGGCGGAACGAAGTAAC  
ACATGTTTGACATCTCCACACGGTGACAGATCAAATATGCCATGAGCACCAGTCCAGAAGTTTTCCAA  
CTATTTATATACTATCCATGCAACCATATAAATTATCAAACATAC

SEQ.I.D. No. 17

Intron 3 antisense sequence (504 bp).

CTGCAAAAAAGAGAGCAGTTTACACAAGAAAAAACTGCTAAATCTCAACAAAAGTATCATGAATTTAA  
TATTAAGGAAGCTATTTGCAACAGAAAGAGTAACTCATGATAATAGAAGGAAATTGTGAAGCAACAGAA  
GGAAGACTTTCTTTATTTCTACAAAATTGCTTTAAGACTATATTTGATGCTTGATAGTACATGTTGAA  
TCCCTCAGCTTCTTTATGTCTATACTTTTTTTATATTTTGAATCTCCTTAGTGAAAATCTTTGCTTTG  
CCACTGACACTCCGGGGGTGTGTCACTTCTCCAAAACCTTGTCTACTTTTTTGAAGACCCAATCAAAC  
AGCTTTTTTAAAGATCAAAAAAATGGCCAGGTGCCACCTAAATGGAGCCACTACTTACTCCCCGGTATG  
CAAAATCTCTAGCAAAGTCAAAGTAGGTATAAACAATTCATCTTCCAAAATAAGGTCAAAGTGCCTAA  
AGCACAACCTTTGGCTGTTAC

SEQ.I.D. No. 18

Intron 4 antisense sequence (146 bp).

CTGCATTGTGGATGAGTTAATTAGAAGCATAACCTTAATAGCAATTAGAACATGTAAGAAAGCCAATGA  
TGCTGCAACATCATGCTTTAATAGGAAAATCTGTTATGATGATGGAACTACTATTTTGTAGTAGACGA  
GGACCTAC

SEQ.I.D. No. 19

Intron 5 antisense sequence (218 bp).

CTGTTTAATTGCTGAAGTAGTAAGTTCTCAAGCACTTATAGAATTGACTCATTTTGTTAAGGGAAAGAG  
TATGGGATCAAGTCCAAATTAGTAAAGACACAATTATTTAACTTTTGCATTTCAAAATGTCTTACATA  
ACAAGACTAGTAAGAACATGAATCGAAATGCCTGTGATGATGGTGTTCAAAATTCAGCTTCAAGGTATG  
AATAACAAAAC

SEQ.I.D. No. 20

Intron 6 antisense sequence (198 bp).

CTGTATCCAGCAGACATAATAGGAGTGAACATAAAAATGTCACTGGATAAATAACTTATCATGATATTC  
AGCGGCTACCAATATTCTGAAGGCCCATGGCGAAAATAAGTACTTTTATACTTTTCAGGACGTATATATT  
TGGATTCTATCTAACAATTGTTCTGAGAATTATTTAGTTGTAGAAATAAATTTAAAATAC

SEQ.I.D. No. 21

Intron 7 antisense sequence (208 bp).

CTGTGGTTAGAAGCTAAAAGTGAATAGATGAGAAAAATTACCTCCAAATAAGAGGGATATTGAAAAAGA  
AACACAATGCATGAAAAGAATAAACAAATGATAACGAGAAAAATTGAATAATCCATCAGAACCCTGGTT  
ACCTCACAAAGAGTGAGATTTTCCGTGGCTAACCTATATGAACCTTAAATGCAATAGAAACAGACAAAC

SEQ.I.D. No. 22

Intron 8 antisense sequence (293 bp).

CTGTACAAGTTCATCAAACATTTTACAATTACTCCAAAACAGACACACTTGCAAACCTCTATACAGTAAT  
CTTCTATACTACAAAAAAGTAAACAATGTTTTTTTAAAGATGACATTTGTTCTCAGCAACATAATAGAA  
ATCCCTAGACAATGGAAACATTCATCATGTTGTTTTCTCTATGTTTCAACCCCTTTGATGTTCAACAG  
TTCAGGTCATTTTGAGGAATGAATCTTGTTCAAGTAAGCCAACTAATTGTAATTATCACAAAATATCT  
AAAGATGTAAGACATAC

SEQ.I.D. No. 23

Intron 9 antisense sequence (376 bp).

CTGCATTTTCATCATGAGGGGGAGGAAAGACGGAGAAATATAGATATCAGATTTAGACCATTTCAATTAG  
TATCACTTCATTGTAAAGAAAAGGTAAGTATCCAACAAATATAGCAGGCTGTGGATTGGTAGCCTGAAA  
CTATAGCTTCAAAGAATCAACTTAAGCTGCTCATCAAGGCCTTAGTGGTAGAAATGAGGCGGTAATAAG  
TGTAATGAATCTAATACTTGGATCTCGAAACAAAATCAGAAATTCGGTTGGAAAATAAGTAGAACAA

GATGAAATGAGCTATCATCCCCAGAACCAAGTAGACTTCCAAGTAAGCAATCTAAAAATTACTAGATTA  
TTTAACAAGCTGCGATTCAAATACTTGAAC

SEQ.I.D. No. 24

Intron 10 antisense sequence (172 bp).

CTGCAAAGTGAAGTAACTAATCAGTACAGCTATTACCGAATTTGACCAGCTATTGGATTAAATAATATG  
AAATCCATCATCAAGAAATGGAAGGTAAAAAGGTTTCTACTTGTCTTGGATAGAATTAAAGCACTTCA  
TAAACCCAACTTTCACTTTAGATGATTTTAC

SEQ.I.D. No. 25

Intron 11 antisense sequence (145 bp).

CTGTTTTTCGTCATGCGAGGATCAGAAAAAGAGTTAAATTAGACAATGTGAAAATGATTTGTTTCAGTT  
ACTTCTCCATAAACTTGTTTCAGTACATTAAAAACAAGCAGAGCAATAATTTTCATGGATAAGTAAACA  
TATATAC

SEQ.I.D. No. 26

Intron 12 antisense sequence (242 bp).

CTGTCATGAGAACAGATTGTATGTCAGCATGAAGACAAAGATCATCAATAAACAGTTTTCTCCTTTTGT  
AATTAGCTAAACAACGCAGGGGGAGGGCAGGAGGCTCAAACACTTCCGAAGTCAGACAGTCGGATATCT  
TATACAATAAAGATGGATGAGACAATTACAGTTCTTTTTTGGTGAGAGAACTGTACCCTACATCTGTTA  
TCTTATTATCAAAAGTTATTCAAGCAAATCCTTAC

SEQ.I.D. No. 27

Intron 13 antisense sequence (797 bp).

CTTCACAAACAAGGAGAAGAAGAAGCAAAAAGAAAGATGAATATAGTTAGCTTAGTTCAATATAAAAAA  
TTTCTCTCCAAGCTATTTTTCTGCTAGCAAAATTCATTAGTTATTTAACTTTTCTATACATAAAGCTGC  
ACAAAGAAATAGTAGTACATTTTTTTGACTTGCACAAAATAACTGTGTTGTCCATTTTCTGACATGTGT  
TCATCTACATGCACTGTTTCAACAACAACAACACTTTCAGTCCCAACAAGTTGGGTGCTTTAGCTAC  
ACATGTTGCTTTCACCTCTGTTACTTCTTTTTGGACTTTTTTCTTGAGCCAAGGGTCTATTGAAAAAA  
TCCTCTCTACCTCTGAGATAGGAGTAAGTTTTGCATACACTCTACCCTCCCCCTGAAACCACTTTGTGG  
GACTACACGAGGTATGTTGTTGTTGATGTTAGCGCAGACACCAAAGGTGGACATTATATGACTATTCCT  
AGCTTTACTTCAGGGCGGTTTTAAGTTCCCATCAACTTCATTTTTGATCATTTACCTAAGTTTATGCAG  
GTGCAAGCTACATGCACTGGTTTTAGGGAAAAAGAGGATAGAGAAGAATTTTTTGGCATCCTTTTGT  
TGTAACAGTAAGATGCCAAAGTAGACCTTATTACGGCTATTCTACCTTTCAAATTAGTAGTTCAGAG  
GACTTAACTGGCGATTGTGGCGGTAATCAATAGTTAACTTCTATCGCATTCAAATAACTATGAACAAAA  
CCACAATAAAAAGGGAGGTCACACGGCAAGAAGTGTAC

SEQ.I.D. No. 28

Antisense DNA sequence of the SBE gene promoter region.

CGAATGGGTTTTGATAAACTTTGAAATTAATTTCCATTGATTAAATTATGGTACTTTGC	60
TTCAGCTGCTGCCTTCTTGATGTTCTGATTCTTGATTTCCTCATTTTAGTGGCTTTT	120
TAAAAAACATTATGACCCCTTTTGTTAGTCCTCCCTTTCTGAATATTTCACTCAGACCC	180
CATTAGTTTCGAAATTGAAGTAAACATATTTTTTTTAGTATTGTAGTTTTTTTATATTT	240
CTACTTACTTACTCGTTATACAATTTCTATTTAGGTAATCTAATCGACTTTTTGTATACA	300
TAATACATGTATTTTTGGTAAAGAGTTTTTACTTTCTCCTAGTGGTAAGGCAGATATAG	360
TTAAGGATTTATTGACCTAATATGAACGCCAATAATTTTATATTTGTATATACGTATAT	420
TTAAAGTTTACTAGATATGTATAAATAAGATATTTAAATTTAATTATAAATACAAATG	480
ATTATGGTAAATTTTGACCTCAAATTTAAATATTTAAATCAAGATTTGTCACTACTT	540
ATATATATCTTGTTGTAAATCCCTTTTAATCAAGTTGTGAGTTTACAAATATTCGTTGGT	600
TAGGCTAAAAAATAAGCTATAAAGATCAAGTATAAAATTATGCATTTTCTGCATTTAA	660
TTTGAAAAATATGTTGGAGCAATCTAAAATTGTTTGTGATTATATAAATAAGTCGTTTT	720
TTGTTTTTAATAATTGATAAACTATTTATTCTGCTTAAAGTTTAGAATGTCAAAAAATA	780
ATTTATTTTAATGACCTTAAATGATTGAATAAGATGTAGACACACTCAATTACAAAGTTA	840
CAATATTAATACACTTGTCTATTGGGTCATGGATTATATCATCTAATATAAATAACATGT	900
CAAATTAAGCTTCTTATAAAGTTCATAGGAATAAGATAAACTTTGTGAATGGCCAAGC	960
ATTTTTCAGAACATCATGGGTGGTATGACAATCAAATTGAACTTATGGGATGAAAAATGA	1020
ATATCATTCAACTAAGAGGGCACAACTTGACATGTTAGAAAGTAAAGCAAATTTAGTAGT	1080
GGGCCAAATAAAAGAAATTAATTTGTCAGTTTATTCTTAACTTTACCTTCTTTGAACTT	1140
CCACGTTATCAAAGGTTACGGTTCATATGAAGGCCATGTGTATCCTTTTTAATTTTGGT	1200
ATTCCGTGTTCAATATCGATTAATTTAAATTGCGATGACAAAATCCTATATTAAAGTATA	1260
AAGTATTTTCTAAACAGACAAGTTCAATACTTTAATTTTACTGAATGCATAAATTTA	1320
CACTATAATAATCCAGTCGCAGTCTACATTACAATAATTAACAATTTTAGCATGAAATG	1380
AAAACTTTAAATTATATGCCATCAAATCACTTAAAGTATACATTTTTTTAATAACTAGT	1440
TCTAATCCCACTTGAAATGAGAGTTATTTAATATCGACCGTTAATTACCATTTTATTAT	1500
TAAATCTGCAACTACAGTCAACTACACCAATGATTTTGCTGATGCCAACTCATAATATAA	1560
TATCCACCGTTCATGTGATTAATTCAATATTTTATATACGTACGTAACAAAAATTACTAA	1620
ATTAACGTTGGATATACCATACCCTAAGCTCTGCCAAATGTCAATGTTCTATCATTAGCT	1680
ATTTTATGCATCTATAATAGATGTTAAATTCATATTCTAAGATTGAACTTAATCATAAA	1740
CTCAAAATTTGTGGTACCTGTCAATGCCTCCAAAGTTGATTGAACATAAACGTTAAGAT	1800
CTGTGTAATGTTCTTTTCCTTGTAATAATGTATGTATGATAATAATAAGAGAACAAA	1860
ATATGGCAAAATAAACACTTTTTTAAACATGTAACCTCAAAACAAGTAATAGGCAAAAGTAC	1920
AGATGACAACACAACACTGTAAACATCATTGAGGAAAACAAAACCATACAACATTTTGA	1980
CTGTAAATGAAGAGTTTGAAAACAAAACTATGTTCAAACCGACGCCAAGCTAACGAAAA	2040
TAGCCATAGAGTTCTAAGAAGCAGATGCAACAGTTCCACGGGTTAGTATCGTCTGTAGTA	2100
GGACCGGTCATGAGAACTCGAAAGAATCTGAAAGGAAGTAATGCATTTGAACCAGTAATT	2160
GGCCATGAT	

SEQ.I.D. No. 29

Genomic SBE gene

ATCATGGCCA	ATTACTGGTT	CAAATGCATT	ACTTCCTTTC	AGATTCTTTC	GAGTTCTCAT	60
GACCGGTCCT	ACTACAGACG	ATACTAACCC	GTGGAACCTGT	TGCATCTGCT	TCTTAGAACT	120
CTATGGCTAT	TTTCGTTAGC	TTGGCGTCGG	TTTGAACATA	GTTTTTGTTT	TCAAACCTCTT	180
CATTACAGT	CAAAATGTTG	TATGGTTTTT	GTTTTCTCA	ATGATGTTTA	CAGTGTGTG	240
TTGTCATCTG	TACTTTTGCC	TATTACTTGT	TTTGAGTTAC	ATGTTAAAAA	AGTGTATTATT	300
TTGCCATATT	TTGTTCTCTT	ATTATTATTA	TCATACATAC	ATTATTACAA	GGAAAAGACA	360
AGTACACAGA	TCTTAACGTT	TATGTTCAAT	CAACTTTTGG	AGGCATTGAC	AGGTACCACA	420
AATTTTGAGT	TTATGATTAA	GTTCAATCTT	AGAATATGAA	TTTAACATCT	ATTATAGATG	480
CATAAAATA	GCTAATGATA	GAACATTGAC	ATTTGGCAGA	GCTTAGGGTA	TGGTATATCC	540
AACGTTAATT	TAGTAATTTT	TGTTACGTAC	GTATATGAAA	TATTGAATTA	ATCACATGAA	600
CGGTGGATAT	TATATTATGA	GTTGGCATCA	GCAAAATCAT	TGGTGTAGTT	GACTGTAGTT	660
GCAGATTTAA	TAATAAAATG	GTAATTAACG	GTCGATATTA	AAATAACTCT	CATTTCAAGT	720
GGGATTAGAA	CTAGTTATTA	AAAAAATGTA	TACTTTAAGT	GATTTGATGG	CATATAATTT	780
AAAGTTTTTC	ATTTTCATGCT	AAAATTGTTA	ATTATTGTAA	TGTAGACTGC	GACTGGAATT	840
ATTATAGTGT	AAATTTATGC	ATTCAGTGTA	AAATTAAAGT	ATTGAACTTG	TCTGTTTTAG	900
AAAATACTTT	ATACTTTAAT	ATAGGATTTT	GTCATGCGAA	TTTAAATTAA	TCGATATTGA	960
ACACGGAATA	CCAAAATTAA	AAAGGATACA	CATGGCCTTC	ATATGAACCG	TGAACCTTTG	1020
ATAACGTGGA	AGTTCAAAGA	AGGTAAAGTT	TAAGAATAAA	CTGACAAATT	AATTTCTTTT	1080
ATTTGGCCCA	CTACTAAATT	TGCTTTACTT	TCTAACATGT	CAAGTTGTGC	CCTCTTAGTT	1140
GAATGATATT	CATTTTTCAT	CCCATAAGTT	CAATTTGATT	GTCATACCAC	CCATGATGTT	1200
CTGAAAAATG	CTTGGCCATT	CACAAAGTTT	ATCTTAGTTC	CTATGAACTT	TATAAGAAGC	1260
TTTAATTTGA	CATGTTATTT	ATATTAGATG	ATATAATCCA	TGACCCAATA	GACAAGTGTA	1320
TTAATATTGT	AACTTTGTAA	TTGAGTGTGT	CTACATCTTA	TTCAATCATT	TAAGGTCATT	1380
AAAATAAATT	ATTTTTTGAC	ATTCTAAAAC	TTTAAGCAGA	ATAAATAGTT	TATCAATTAT	1440
TAAAAACAAA	AAACGACTTA	TTTATAAATC	AACAAACAAT	TTTAGATTGC	TCCAACATAT	1500
TTTTCCAAAT	TAAATGCAGA	AAATGCATAA	TTTTATACTT	GATCTTTATA	GCTTATTTTT	1560
TTTAGCCTAA	CCAACGAATA	TTTGTAAGT	CACAACTTGA	TTAAAAGGGA	TTTACAACAA	1620
GATATATATA	AGTAGTGACA	AATCTTGATT	TTAAATATTT	TAATTTGGAG	GTCAAAATTT	1680
TACCATAATC	ATTTGTATTT	ATAATTAAAT	TTTAAATATC	TTATTTATAC	ATATCTAGTA	1740
AACTTTTAAA	TATACGTATA	TACAAAATAT	AAAATTATTG	GCGTTCATAT	TAGGTCAATA	1800
AATCCTTAAC	TATATCTGCC	TTACCACTAG	GAGAAAAGTAA	AAAACCTCTT	ACCAAAAATA	1860
CATGTATTAT	GTATACAAAA	AGTCGATTAG	ATTACCTAAA	TAGAAATTGT	ATAACGAGTA	1920
AGTAAGTAGA	AATATAAAAA	AACTACAATA	CTAAAAAATA	TATGTTTTAC	TTCAATTTTCG	1980
AACTAATGG	GGTCTGAGTG	AAATATTCAG	AAAGGGGAGG	ACTAACAAAA	GGGTCAATAAT	2040
GTTTTTTTAT	AAAAAGCCAC	TAAATGAGG	AAATCAAGAA	TCAGAACATA	CAAGAAGGCA	2100
GCAGCTGAAG	CAAAGTACCA	TAATTTAATC	AATGGAAATT	AATTTCAAAG	TTTTATCAAA	2160
ACCCATTGCA	GGATCTTTTC	CATCTTTCTC	ACCTAAAAGT	TCTTCAGGGG	TAATTTTTTAC	2220
TAATTTTCATG	TTAATTTCAA	TTATTTTTTAG	CCTTTGCATT	TCATTTTCCA	ATATATCTGG	2280
ATCATCTCCT	TAGTTTTTTA	TTTTATTTTT	TATAATATCA	AATATGGAAG	AAAAATGACA	2340
CTGTAGAGC	CATATGTAAG	TATCATGTGA	CAAATTTGCA	AGGTGGTTGA	GTGTATAAAA	2400
TTCAAAAATT	GAGAGATGGA	GGGGGGGTGG	GGGBARAGAC	AATATTTAGA	AAGAGTGTTT	2460
TAGGAGGTTA	TGGAGGACAC	GGATGAGGGG	TAGAAGGTTA	GTTAGGTATT	TGAGTGTGTT	2520



CTGGCTTATC	CTTTCATACT	AGTAGTCGTG	GAATTATTTG	GGTAGTTTCT	TGTTTTGTTA	2580
TTTGATCTTT	GTTATTCTAT	TTTCTGTTTC	TTGTACTTCG	ATTATTGTAT	TATATATCTT	2640
GTCGTAGTTA	TTGTTCCCTCG	GTAAGAATGC	TCTAGCATGC	TTCCTTTAGT	GTTTTATCAT	2700
GCCTTCTTTA	TATTCGCGTT	GCTTTGAAAT	GCTTTTFACTT	TAGCCGAGGG	TCTATTAGAA	2760
ACAATCTCTC	TATCTCGTAA	GGTAGGGGTA	AAGTCCTCAC	CACACTCCAC	TTGTGGGATT	2820
ACATTGTGTT	TGTTGTTGTA	AATCAATTAT	GTATACATAA	TAAGTGGATT	TTTTACAACA	2880
CAAATACATG	GTCAAGGGCA	AAGTTCTGAA	CACATAAAGG	GTTCAATTATA	TGTCCAGGGA	2940
TATGATAAAA	ATTGTTTCTT	TGTGAAAGTT	ATATAAGATT	TGTTATGGCT	TTTGCTGGAA	3000
ACATAATAAG	TTATAATGCT	GAGATAGCTA	CTGAAGTTTG	TTTTTTCTAG	CCTTTTAAAT	3060
GTACCAATAA	TAGATTCCGT	ATCGAACGAG	TATGTTTTGA	TTACCTGGTC	ATGATGTTTC	3120
TATTTTTTAC	ATTTTTTTGG	TGTTGAACTG	CAATTGAAAA	TGTTGTATCC	TATGAGACGG	3180
ATAGTTGAGA	ATGTGTTCTT	TGTATGGACC	TTGAGAAGCT	CAAACGCTAC	TCCAATAATT	3240
TCTATGAATT	CAAATTCAGT	TTATGGCTAC	CAGTCAGTCC	AGAAATTAGG	ATATGCTGCA	3300
TATACTTGTT	CAATTATACT	GTAAAATTTT	TTAAGTTCTC	AAGATATCCA	TGTAACCTCG	3360
AGAATTTCTT	TGACAGGCTT	CTAGAAATAA	GATATGTTTT	CCTTCTCAAC	ATAGTACTGG	3420
ACTGAAGTTT	GGATCTCAGG	AACGGTCTTG	GGATATTTCT	TCCACCCCAA	AATCAAGAGT	3480
TAGAAAAGAT	GAAAGGGTAT	GTTTGATAAT	TTATATGGTT	GCATGGATAG	TATATAAATA	3540
GTTGAAAAC	TTCTGGACTG	GTGCTCATGG	CATATTTGAT	CTGTGCACCG	TGTGGAGATG	3600
TCAAACATGT	GTTACTTCGT	TCCGCCAATT	TATAATACCT	TAACCTGGGA	AAGACAGCTC	3660
TTTACTCCTG	TGGGCATTTG	TTATTTGAAT	TACAATCTTT	ATGAGCATGG	TGTTTTCACA	3720
TTATCAACTT	CTTTCATGTG	GTATATAACA	GTTTTTAGCT	CCGTTAATAC	CTTCTTCTT	3780
TTTGATATAA	ACTAATGTG	GTGCATTGCT	TGCBKKKATG	AAGCACAGTT	CAGCTATTTT	3840
CGCTGTTTTG	ACCGATGACG	ACAATTCGAC	AATGGCACCC	CTAGAGGAAG	ATGTCAAGAC	3900
TGAAAATATT	GGCCTCCTAA	ATTTGGATCC	AACTTTGGAA	CCTTATCTAG	ATCACTTCAG	3960
ACACAGAATG	AAGAGATATG	TGGATCAGAA	AATGCTCATT	GAAAAATATG	AGGGACCCCT	4020
TGAGGAATTT	GCTCAAGGTA	ACAGCCAAAA	GTTGTGCTTT	AGGCAGTTTG	ACCTTATTTT	4080
GGAAGATGAA	TTGTTTATAC	CTACTTTGAC	TTTGCTAGAG	AATTTTGCAT	ACCGGGGAGT	4140
AAGTAGTGGC	TCCATTTAGG	TGGCACCTGG	CCATTTTTTT	GATCTTTTAA	AAAGCTGTTT	4200
GATTGGGTCT	TCAAAAAAGT	AGACAAGGTT	TTTGAGAAG	TGACACACCC	CCGGAGTGTC	4260
AGTGGCAAAG	CAAAGATTTT	CACTAAGGAG	ATTCAAAATA	TAAAAAAGT	ATAGACATAA	4320
AGAAGCTGAG	GGGATTCAAC	ATGTACTATA	CAAGCATCAA	ATATAGTCTT	AAAGCAATTT	4380
TGTAGAAATA	AAGAAAGTCT	TCCTTCTGTT	GCTTCACAA	TTCCTTCTAT	TATCATGAGT	4440
TACTCTTTCT	GTTCGAAATA	GCTTCCTTAA	TATTAAATTC	ATGATACTTT	TGTTGAGATT	4500
TAGCAGTTTT	TTCTTGTA	AACTGCTCTC	TTTTTTTGCA	GGTTATTTAA	AATTTGGATT	4560
CAACAGGGAA	GATGGTTGCA	TAGTCTATCG	TGAATGGGCT	CCTGCTGCTC	AGTAGGTCCT	4620
CGTCTACTAC	AAAATAGTAG	TTTCCATCAT	CATAACAGAT	TTTCCTATTA	AAGCATGATG	4680
TTGCAGCATC	ATTGGCTTTC	TTACATGTTT	TAATTGCTAT	TAAGGTTATG	CTTCTAATTA	4740
ACTCATCCAC	AATGCAGGGA	AGCAGAAGTT	ATTGGCGATT	TCAATGGATG	GAACGGTTCT	4800
AACCACATGA	TGGAGAAGGA	CCAGTTTGGT	GTTTGGAGTA	TTAGAATTCC	TGATGTTGAC	4860
AGTAAGCCAG	TCATTCCACA	CAACTCCAGA	GTTAAGTTTC	GTTTTCAAACA	TGGAATGGA	4920
GTGTGGGTAG	ATCGTATCCC	TGCTTGATA	AAGTATGCCA	CTGCAGACGC	CACAAAGTTT	4980
GCAGCACCAT	ATGATGGTGT	CTACTGGGAC	CCACCACCTT	CAGAAAGGTT	TTGTTATTCA	5040
TACCTTGAAG	CTGAATTTTG	AACACCATCA	TCACAGGCAT	TTCGATTCAT	GTTCTTACTA	5100
GTCTTGTTAT	GTAAGACATT	TTGAAATGCA	AAAGTTAAAA	TAATTGTGTC	TTTACTAATT	5160
TGGACTTGAT	CCCATACTCT	TTCCCTTAAC	AAAATGAGTC	AATTCTATAA	GTGCTTGAGA	5220

ACTTACTACT	TCAGCAATTA	AACAGGTACC	ACTTCAAATA	CCCTCGCCCT	CCCAAACCCC	5280
GAGCCCCACG	AATCTATGAA	GCACATGTCG	GCATGAGCAG	CTCTGAGCCA	CGTGTAAT	5340
CGTATCGTGA	GTTTGCAGAT	GATGTTTTAC	CTCGGATTAA	GGCAAATAAC	TATAATACTG	5400
TCCAGTTGAT	GGCCATAATG	GAACATTCTT	ACTATGGATC	ATTTGGATAT	CATGTTACAA	5460
ACTTTTTTGC	TGTGAGCAGT	AGATATGGAA	ACCCGGAGGA	CCTAAAGTAT	CTGATAGATA	5520
AAGCACATAG	CTTGGGTTTA	CAGGTTCTGG	TGGATGTAGT	TCACAGTCAT	GCAAGCAATA	5580
ATGTCACTGA	TGGCCTCAAT	GGCTTTGATA	TTGGCCAAGG	TTCTCAAGAA	TCCTACTTTC	5640
ATGCTGGAGA	GCGAGGTAC	CATAAGTTGT	GGGATAGCAG	GCTGTTCAAC	TATGCCAATT	5700
GGGAGGTTCT	TCGTTTCCTT	CTTTCCAAC	TGAGGTGGTG	GCTAGAAGAG	TATAACTTTG	5760
ACGGATTTTCG	ATTTGATGGA	ATAACTTCTA	TGCTGTATGT	TCATCATGGA	ATCAATATGG	5820
GATTTACAGG	AACTATAAT	GAGTATTTCA	GCGAGGCTAC	AGATGTTGAT	GCTGTGGTCT	5880
ATTTAATGTT	GGCCAATAAT	CTGATTACACA	AGATTTTCCC	AGATGCAACT	GTTATTGCCG	5940
AAGATGTTTC	TGGTATGCCG	GGCCTTGGCC	GGCCTGTTTC	TGAGGGAGGA	ATTGGTTTTG	6000
TTTACCGCCT	GGCAATGGCA	ATCCCAGATA	AGTGGATAGA	TTATTTAAAG	AATAAGAATG	6060
ATGAAGATTG	GTCCATGAAG	GAAGTAACAT	CGAGTTTGAC	AAATAGGAGA	TATACAGAGA	6120
AGTGATATAGC	ATATGCGGAG	ACCCATGATC	AGGTATTTTA	AATTTATTTT	TACAACTAAA	6180
TAATTCTCAG	AACAATTGTT	AGATAGAATC	CAAATATATA	CGTCCTGAAA	GTATAAAAGT	6240
ACTTATTTTC	GCCATGGGCC	TTCAGAATAT	TGGTAGCCGC	TGAATATCAT	GATAAGTTAT	6300
TTATCCAGTG	ACATTTTTAT	GTTCACTCCT	ATTATGTCTG	CTGGATACAG	TCTATTGTTG	6360
GTGACAAGAC	CATTGCATTT	CTCCTAATGG	ACAAAGAGAT	GTATTCTGGC	ATGTCTTGCT	6420
TGACAGATGC	TTCTCCTGTT	GTTGATCGAG	GAATTGCGCT	TCACAAGGTT	TGTCTGTTTC	6480
TATTGCATTT	TAAGGTTTAT	ATAGGTTAGC	CACGGAAAT	CTCACTCTTT	GTGAGGTAAC	6540
CAGGGTTCTG	ATGGATTATT	CAATTTTCTC	GTTTATCATT	TGTTTATTCT	TTTCATGCAT	6600
TGTGTTTCTT	TTTCAATATC	CCTCTTATTT	GGAGGTAATT	TTTCTCATCT	ATTCACTTTT	6660
AGCTTCTAAC	CACAGATGAT	CCATTTTTTC	ACAATGGCCT	TGGGAGGAGA	GGGGTACCTC	6720
AATTTTCATGG	GTAACGAGGT	ATGTCTTACA	TCTTTAGATA	TTTTGTGATA	ATTACAATTA	6780
GTTTGGCTTA	CTTGAACAAG	ATTCATTCTC	CAAAATGACC	TGAACTGTTG	AACATCAAAG	6840
GGGTGAAAC	ATAGAGGAAA	ACAACATGAT	GAATGTTTCC	ATTGTCTAGG	GATTTCTATT	6900
ATGTTGCTGA	GAACAAATGT	CATCTTAAAA	AAAACATTGT	TTACTTTTTT	GTAGTATAGA	6960
AGATTACTGT	ATAGAGTTTG	CAAGTGTGTC	TGTTTTGGAG	TAATTGTGAA	ATGTTTGATG	7020
AACTTGTA	GTTTGGCCAT	CCTGAGTGGA	TTGACTTCCC	TAGAGAGGGC	AATAATTGGA	7080
GTTATGACAA	ATGTAGACGC	CAGTGAACC	TCGCGGATAG	CGAACACTTG	AGATACAAGG	7140
TTCAAGTATT	TTGAATCGCA	GCTTGTTAAA	TAATCTAGTA	ATTTTTAGAT	TGCTTACTTG	7200
GAAGTCTACT	TGGTCTGGG	GATGATAGCT	CATTTTCATCT	TGTTCTACTT	ATTTTCCAAC	7260
CGAATTTCTG	ATTTTTGTTT	CGAGATCCAA	GTATTAGATT	CATTTACACT	TATTACCGCC	7320
TCATTTCTAC	CACTAAGGCC	TTGATGAGCA	GCTTAAGTTG	ATTCTTTGAA	GCTATAGTTT	7380
CAGGCTACCA	ATCCACAGCC	TGCTATATTT	GTTGGATACT	TACCTTTTCT	TTACAATGAA	7440
GTGATACTAA	TTGAAATGGT	CTAAATCTGA	TATCTATATT	TCTCCGTCTT	TCCTCCCCCT	7500
CATGATGAAA	TGCAGTTTAT	GAATGCATTT	GATAGAGCTA	TGAATTCGCT	CGATGAAAAG	7560
TTCTCATTC	TCGCATCAGG	AAAACAGATA	GTAAGCAGCA	TGGATGATGA	TAATAAGGTA	7620
AAATCATCTA	AAGTTGAAAG	TGTTGGGTTT	ATGAAGTGCT	TTAATTCTAT	CCAAGGACAA	7680
GTAGAAACCT	TTTTACCTTC	CATTTCTTGA	TGATGGATTT	CATATTATTT	AATCCAATAG	7740
CTGGTCAAAT	TCGGTAATAG	CTGTACTGAT	TAGTTACTTC	ACTTTGCAGG	TTGTTGTGTT	7800
TGAACGTGGT	GACCTGGTAT	TTGTATTCAA	CTTCCACCCA	AAGAACACAT	ACGAAGGGTA	7860
TATATGTTTT	ACTTATCCAT	GAAATTATTG	CTCTGCTTGT	TTTTAATGTA	CTGAACAAGT	7920

TTTATGGAGA	AGTAACTGAA	ACAAATCATT	TTCACATTGT	CTAATTTAAC	TCTTTTTTCT	7980
GATCCTCGCA	TGACGAAAAC	AGGTATAAAG	TTGGATGTGA	CTTGCCAGGG	AAGTACAGAG	8040
TTGCACTGGA	CAGTGATGCT	TGGGAATTTG	GTGGCCATGG	AAGAGTAAGG	ATTGCTTGA	8100
ATAACTTTTG	ATAATAAGAT	AACAGATGTA	GGGTACAGTT	CTCTACCAA	AAAGAACTGT	8160
AATTGTCTCA	TCCATCTTTA	GTTGTATAAG	ATATCCGACT	GTCTGAGTTC	GGAAGTGTTT	8220
GAGCCTCCTG	CCCTCCCCCT	GCGTTGTTTA	GCTAATTCAA	AAAGGAGAAA	ACTGTTTATT	8280
GATGATCTTT	GTCTTCATGC	TGACATACAA	TCTGTTCTCA	TGACAGACTG	GTCATGATGT	8340
TGACCATTTC	ACATCACCAG	AAGGAATACC	TGGAGTTCCA	GAAACAAATT	TCAATGGTCG	8400
TCCAAATTCC	TTCAAAGTGC	TGTCTCCTGC	GCGAACATGT	GTGGTACAGT	TCTTGCCGTG	8460
TGACCTCCCT	TTTTATTGTG	GTTTTGTTCA	TAGTTATTTG	AATGCGATAG	AAGTTAACTA	8520
TTGATTACCG	CCACAATCGC	CAGTTAAGTC	CTCTGAACTA	CTAATTTGAA	AGGTAGGAAT	8580
AGCCGTAATA	AGGTCTACTT	TTGGCATCTT	ACTGTTACAA	AACAAAAGGA	TGCCAAAAAA	8640
ATTCTTCTCT	ATCCTCTTTT	TCCCTAAACC	AGTGCATGTA	GCTTGCACCT	GCATAAACTT	8700
AGGTAAATGA	TCAAAAATGA	AGTTGATGGG	AACTTAAAAC	CGCCTGAAG	TAAAGCTAGG	8760
AATAGTCATA	TAATGTCCAC	CTTTGGTGTC	TGCGCTAACA	TCAACAACAA	CATACCTCGT	8820
GTAGTCCCAC	AAAGTGGTTT	CAGGGGGAGG	GTAGAGTGTA	TGCAAACTT	ACTCCTATCT	8880
CAGAGGTAGA	GAGGATTTTT	TCAATAGACC	CTTGGCTCAA	GAAAAAAGT	CCAAAAAGAA	8940
GTAACAGAAG	TGAAAGCAAC	ATGTGTAGCT	AAAGCGACCC	AAC TTGTTTG	GGACTGAAGT	9000
AGTTGTTGTT	GTTGAAACAG	TGCATGTAGA	TGAACACATG	TCAGAAAATG	GACAACACAG	9060
TTATTTTGTG	CAAGTCAAAA	AAATGTACTA	CTATTTCTTT	GTGCAGCTTT	ATGTATAGAA	9120
AAGTTAAATA	ACTAATGAAT	TTTGCTAGCA	GAAAAATAGC	TTGGAGAGAA	ATTTTTTATA	9180
TTGAACTAAG	CTAACTATAT	TCATCTTTCT	TTTTGCTTCT	TCTTCTCCTT	GTTTGTGAAG	9240
GCTTATTACA	GAGTTGATGA	ACGCATGTCA	GAAACTGAAG	ATTACCAGAC	AGACATTTGT	9300
AGTGAGCTAC	TACCAACAGC	CAATATCGAG	GAGAGTGACG	AGAACTTAA	AGATTCGTTA	9360
TCTACAAATA	TCAGTAACAT	TGACGAACGC	ATGTCAGAAA	CTGAAGTTTA	CCAGACAGAC	9420
ATTTCTAGTG	AGCTACTACC	AACAGCCAAT	ATTGAGGAGA	GTGACGAGAA	ACTTAAAGAT	9480
TCGTTATCTA	CAAATATCAG	TAACATTGAT	CAGACTGTTG	TAGTTTCTGT	TGAGGAGAGA	9540
GACAAGGAAC	TTAAAGATTG	ACCGTCTGTA	AGCATCATTA	GTGATGTTGT	TCCAGCTGAA	9600
TGGGATGATT	CAGATGCAAA	CGTCTGGGGT	GAGGACTAGT	CAGATGATTG	ATCGACCCCT	9660
CTACCGATTG	GTGATCGCTA	TCCTTGCTCT	CTGAGAAATA	GGTGAGGCGA	AACAAAAAAT	9720
AATTTGCATG	ATAAAAAGTC	TGATTTTATG	ATCGCTATCC	TCGCTCTCTG	AGAAAGAAGC	9780
GAAACAAAGG	CGACTCCTGG	ACTCGAATCT	ATAAGATAAC	AAAGGCGACT	CCTGGGACTC	9840
GAATCTATAA	GATAACAAAG	GCAATTCCAA	GACTTGAATC	TATAAAAAAT	TTAGTTAAGA	9900
ATGATTAACG	TCCGATCCTA	ATTGGAATCG	AGGCATCTTA	CCACTCCATT	GATAATTATA	9960
TAAGTCAATA	AGTCATATAA	WAGTATTAAA	AATAAATTG	ACTTGATCGG	TCTATCAAAA	10020
ATMAGATMAA	ATTGTGTTCA	TATGTAACAT	TTTTGTTGTC	ACAATTAGCT	TAATTACATC	10080
TTTCATGTGC	AATAACAAAG	AAATGATAGG	AATTTAGAGA	TTCCAATTTT	TTTGTTGCCA	10140
CAATTAACCT	AATTACATCT	TTCAATTTGCA	ATAACAAAGA	AATGATAGGA	ATTTAGAGAT	10200
CCAGTGTCAG	TACACAACCT	AGGCCAACAT	CGAAAGCATA	ACTGTAAACT	CATGCATGAA	10260
GAAATCAGTC	GTAAAAATGA	ATAAATGCGA	CATAAAAAACA	AATTGCATGT	ATCATTAAATG	10320
TGACTTAACT	ACAAGTAAAA	ATAAATTTAA	CAAATGTAAC	TTAACTACAA	GTAAAAATAA	10380
ATTGCTTCTA	TCATTAACAA	ACAAACAGAA	TTAAAAAGAA	AAAAACATAC	TAAATCTTAC	10440
CGTCATTGCA	TAAAAAATAA	TACCAAATTC	ATAATGCAAG	GAAAACGAAA	CGCGTCTCTGA	10500
TCGGGTATCA	ACGATGAAAT	GGACCAGTTG	GATCGACTGC	CTGCACAACG	TTAGGTATGC	10560
CAAAAAAAG	AACACGATCC	TTTGCACCCG	TTGATGATT	ATCAGTATGT	TCACAAAAAA	10620

AACTTAAGTT CATCCCAGTG TACAACAGCC CCAACATCTG CCCCAGGTAA CAAAAACAA	10680
CCAATTTATC TTATTCTTAT CTGCCACAAA ATAATCGGTT TCACACTATT CTCTTGTTAT	10740
ACAAAATTGA CAAGTAGGAA GGAGAGGAGT CATCCAAATA AACGGTGCAC GTTCTTTGAG	10800
AAAAGTCTTA TTTTTCGTAA GATCCAATTT CAACAACTT TTCTTCAAGT CAAAATTCCT	10860
GATAGTGTAT CTCCTCTCGA CGACCTCTTG CATTGAACGA TCTCCGCTTA TCATGAAAAG	10920
TTGCTTGGAT AACAAGTATT GCAAGGGGGG GACAGTAGCT ATTAAGTTAG TCGGCCCAAG	10980
GAAATGGAGG AGTGATAGTC TCGAATATTA TTCACCTCTT TAGCATTACC CGGTCTGGCT	11040
TTAAGGAGTT ACGTCTTTTA CGCTCGCCAA TTCTTTTTTT TAGAATGGTT GGTGTCAAAA	11100
TCGCGAGTTG TGGAAGGTTT AAGTTACTCG ATTCGTGATT TTCAAGTATG AGTGGTGAGA	11160
GAGATTTCGAT ATTTTCACGA GGTGTATTCT AGGTCTAGTA GAACGAAGGG TGTCACTAAT	11220
GAAAGTTTCA AGAGTTCATC ATCATCTTCT TCTAGTAGAT TTTCGCTTTC AAATGAGTAT	11280
GAAAATTCTT CCTCTTTTCT ATTGATTTTC TTCATTGTTT TCTTCATTGT TGTGGTTGTT	11340
ATTGAAAAGA AAGAAAATTT ATAACAGAAA AAGATGTCAA AAAAAAGGTA AAATGAAAGA	11400
GTATCATATA CTAAAGAGT TGCCTAGAGA TAAGTCAAAA GAAACAGAAT TATAGTAATT	11460
TCAGCTAAGT TAGAATTC	11478

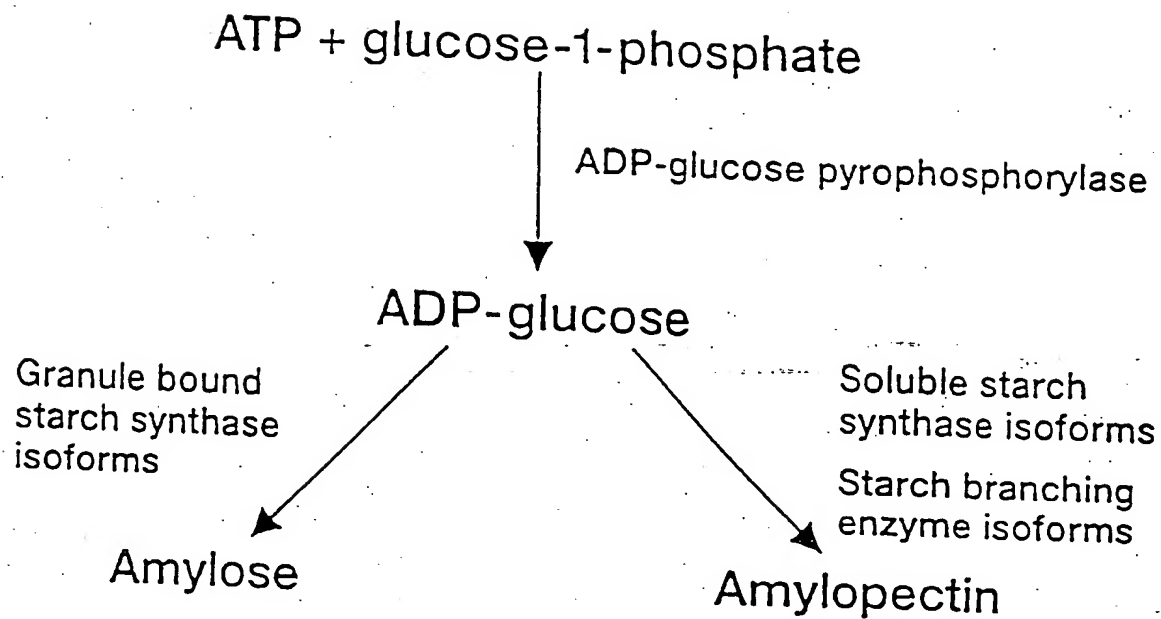
## CLAIMS

1. A method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.
2. A method according to claim 1 wherein starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.
3. A method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.
4. A method according to any one of claims 1 to 3 wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence.
5. A method according to any one of the preceding claims wherein the enzymatic activity is reduced or eliminated.
6. A method according to any one of the preceding claims wherein the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

7. A method according to any one of the preceding claims wherein the nucleotide sequence codes for all of at least one intron in a sense orientation.
8. A method according to any one of the preceding claims wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.
9. A method according to any one of the preceding claims wherein the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.
10. A sense sequence comprising the nucleotide sequence as defined in claim 8 or a variant, derivative or homologue thereof.
11. A promoter having a sequence shown as SEQ.I.D. No. 14, or a variant, derivative or homologue thereof.
12. A promoter according to claim 11 in combination with a gene of interest ("GOI").
13. A construct capable of comprising or expressing the invention according to any one of claims 10 to 12.
14. A vector comprising or expressing the invention according to any one of claims 10 to 13.
15. A combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

16. A cell, tissue or organ comprising or expressing the invention according to any one of claims 10 to 15.
- 5 17. A transgenic starch producing organism comprising or expressing the invention according to any one of claims 10 to 16.
18. A transgenic starch producing organism according to claim 17 wherein the organism is a plant.
- 10 19. A starch obtained from the invention according to any one of the preceding claims.
20. pBEA11 (NCIMB 40754).
- 15 21. An intron nucleotide sequence that is obtainable from  $\lambda$ -SBE 3.2 (NCIMB 40751) or  $\lambda$ -SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.
- 20 22. A method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence; wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon
- 25 sequence normally associated with the intron.

1/17



Reducing end



Reducing end

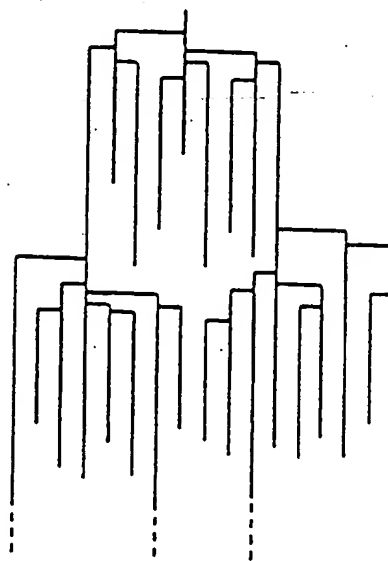


Fig 1



2/17

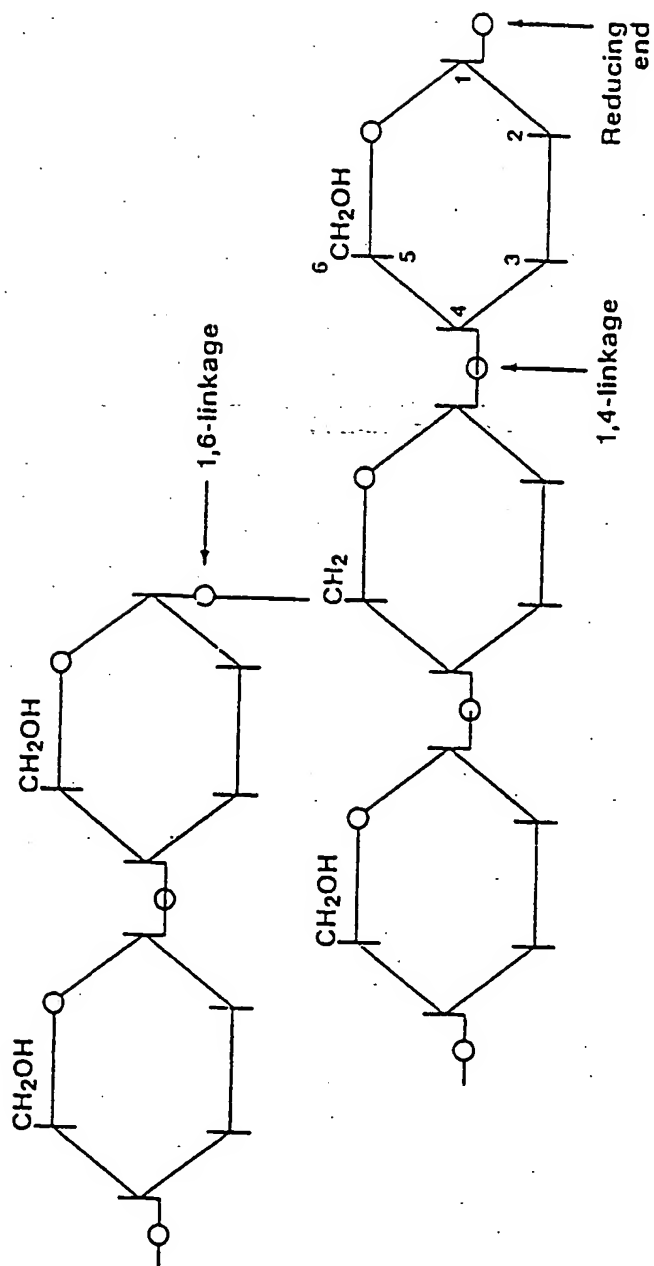


Fig 2

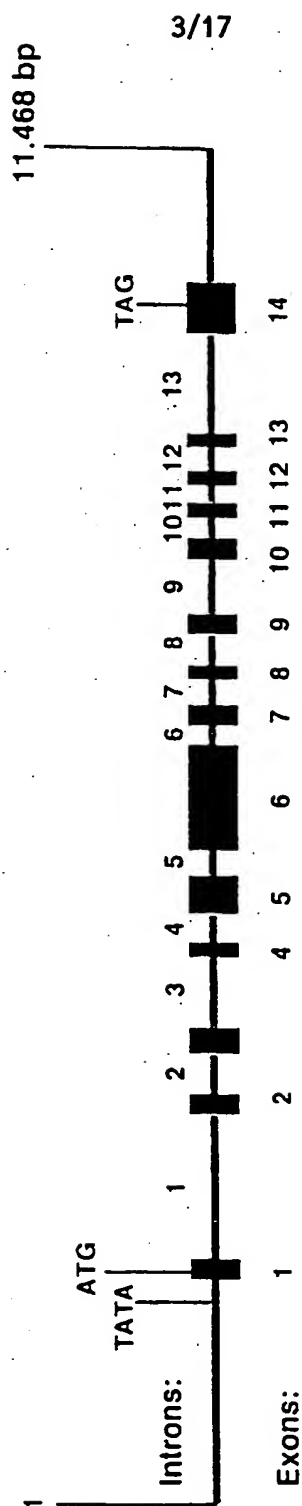


Fig 3

4/17

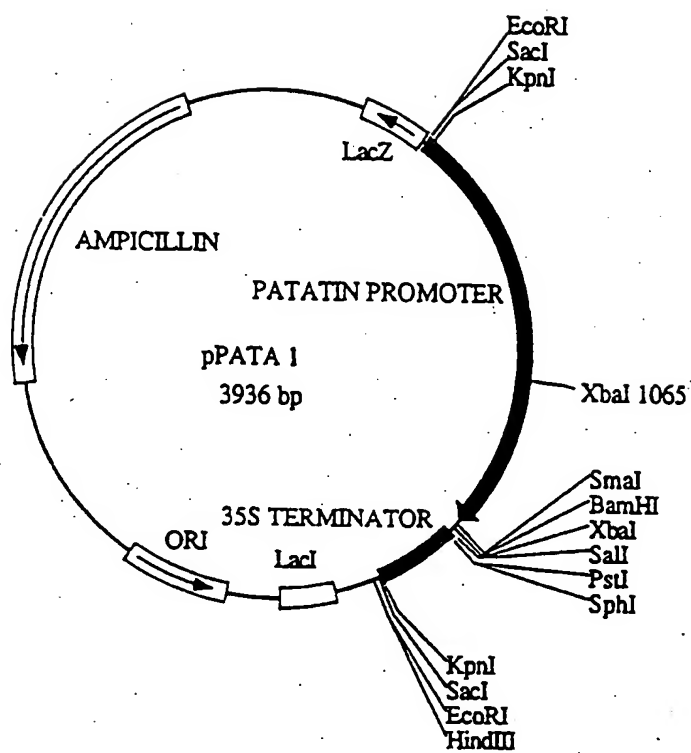


Fig 4

5/17

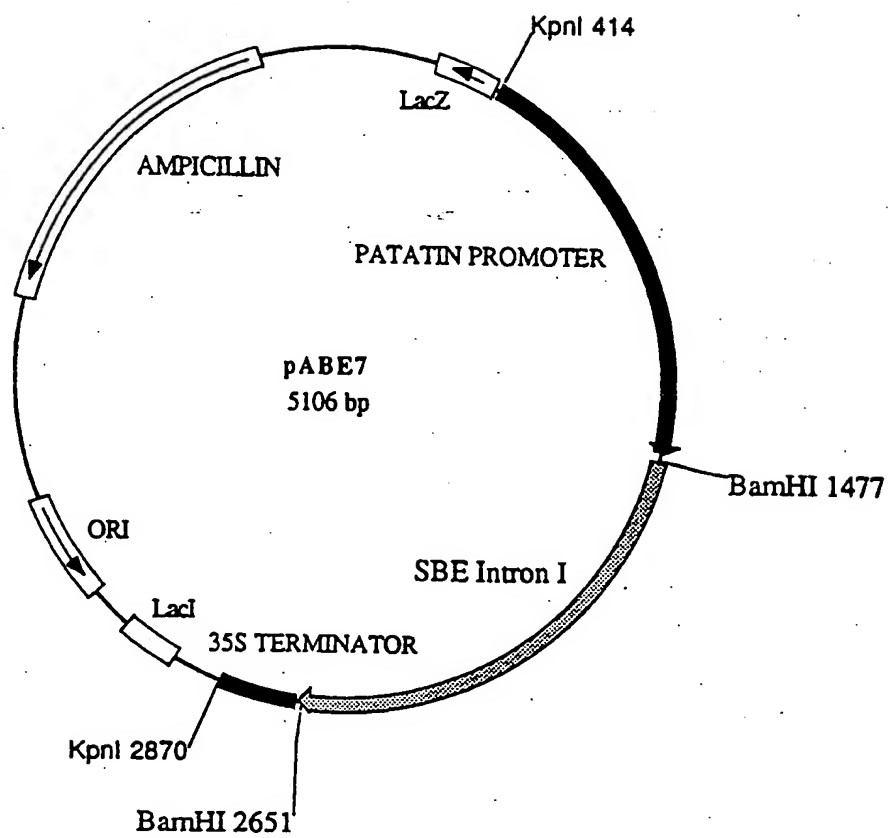


Fig 5

6/17

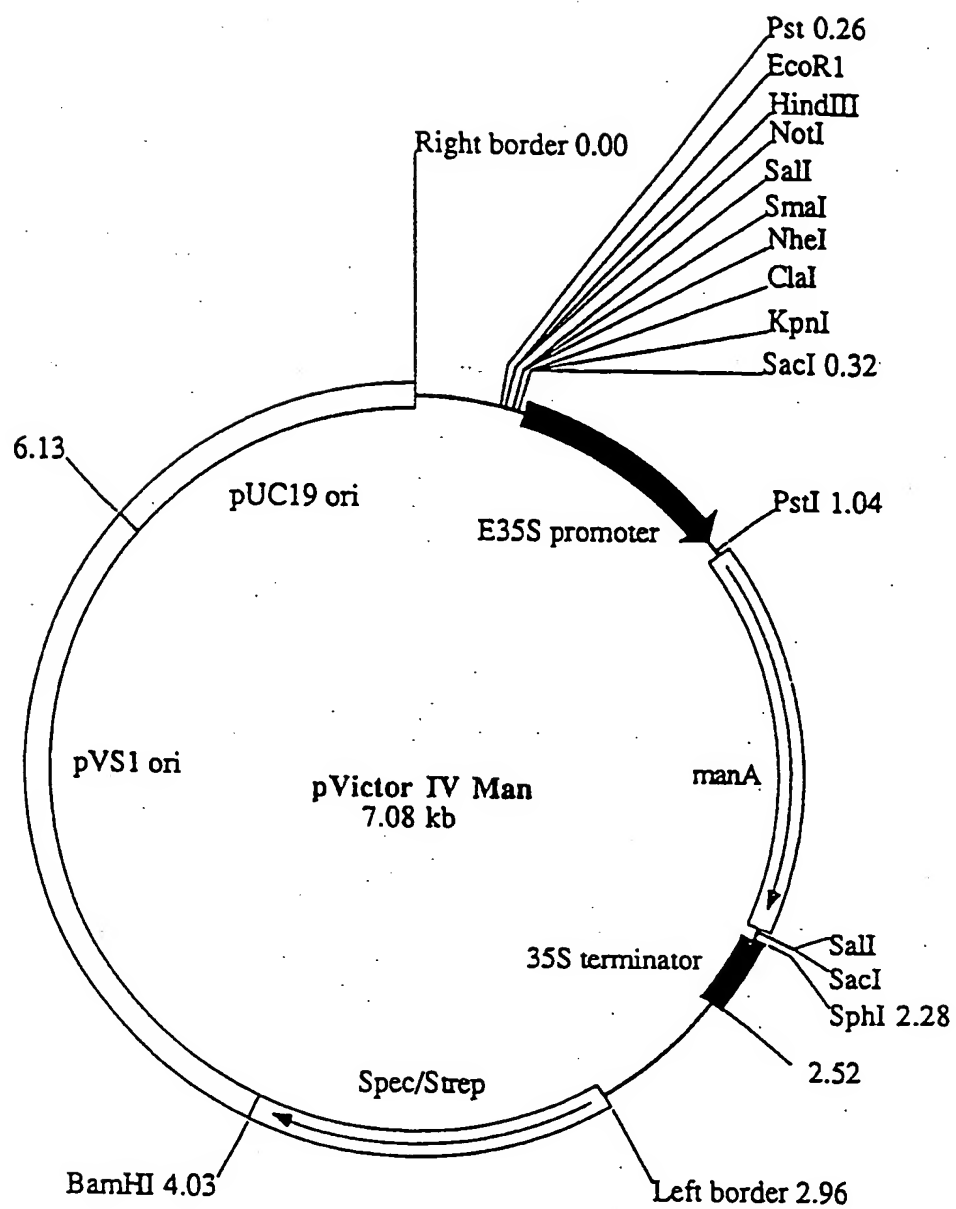


Fig 6

7/17

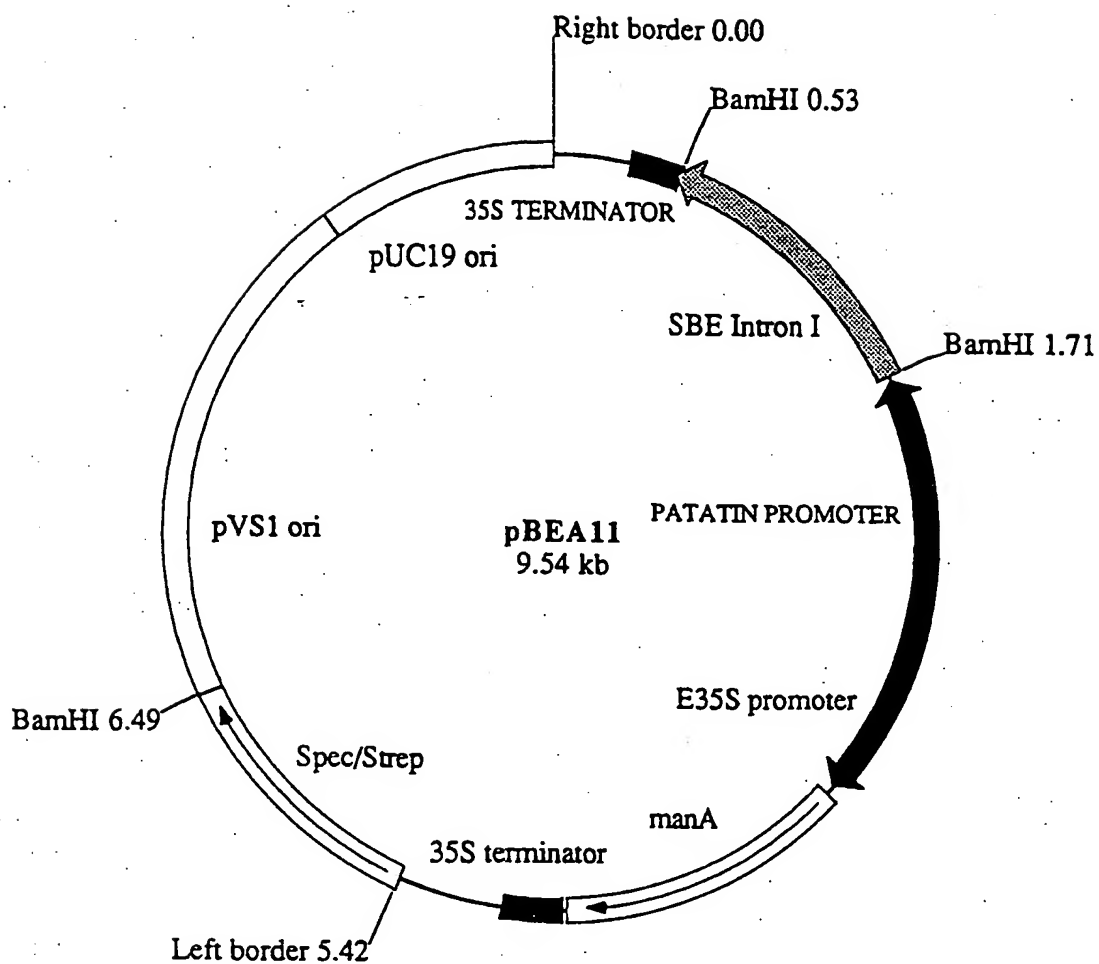


Fig 7

8/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTCAGATTCTTTCGAGTTCTCAT						60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAACGTGTCATCTGCTTCTTAGAACT						120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTGTGTTTCAAACCTCTT						180
CATTTACAGTCAAAATGTTGTATGGTMTTGTGTTTCTCAATGATGTTTACAGTGTGTG						240
TTGTCATCTGTACTTTTGCCTATTACTTGTGTTTGTGTTTACATGTTAAAAAAGTGTATT						300
TTGCCATATTTTGTGTTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA						360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA						420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG						480
CATAAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC						540
AACGTTAATTTAGTAATTTTGTGTACGTACGTATATGAAATATTGAATTAATCACATGAA						600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATGGTGTTAGTTGACTGTAGTT						660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAAAAATAACTCTCATTTCAAGT						720
GGGATTAGAACTAGTTATTAAAAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT						780
AAAGTTTTTCATTTTCATGCTAAAATTGTTAATTATTGTAATGTAGACTGCGACTGGAATT						840
ATTATAGTGTAATTTTATGCATTCAGTGTAATTAAGTATTGAACTTGTCTGTTTATG						900
AAAATACTTTTATACTTTAATATAGGATTTTGTGTCATGCGAATTTAAATTAATCGATATTGA						960
ACACGGAATACCAAAATTAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG						1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAAGATAAACTGACAAATTAATTTCTTTT						1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT						1140
GAATGATATTCATTTTTCATCCCATAAGTTCAATTTGATTGTCATACCACCATGATGTT						1200
CTGAAAAATGCTTGGCCATTACAAAGTTTATCTTAGTTTCTATGAACTTTATAAGAAGC						1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA						1320
TTAATATTGTAACTTTGTAAATTGAGTGTGTCTACATCTTATTCAATCATTTAAGGTCATT						1380
AAAATAAATTATTTTTTGACATTCTAAACTTTAAGCAGAATAAATAGTTTATCAATTAT						1440
TAAAAACAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT						1500

Fig 8

9/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTTT						1560
TTTAGCCTAACCAACGAATATTTGTAACTCACAACCTGATTAAAAGGGATTTACAACAA						1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAAATTTGGAGGTCAAATTT						1680
TACCATAATCATTTGTATTTATAATTAATTTTAAATATCTTATTTATACATATCTAGTA						1740
AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTCATATTAGGTCAATA						1800
AATCCTTAACTATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA						1860
CATGTATTATGTATACAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA						1920
AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAATATGTTTTACTTCAATTTTCG						1980
AACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT						2040
GTMTTMTATATAAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA						2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAATTAATTTCAAAGTTTTATCAAA						2160
ACCCATTGAGGATCTTTTCCATCTTTCTCACCTAAAGTTTCTTCAGGGgtaatttttac						2220
P I R G S F P S F S P K V S S G						
taatttcattgtaatttcattatatttttagcctttgcatttcattttccaatatatctgg						2280
atcatctccttagttttttattttattttttataatatcaaatatggaagaaaaatgaca						2340
cttgtagagccatatgtaagtatcatgtgacaaatttgcaagggtggttgagtgtataaaa						2400
ttcaaaaattgagagatggagggggggtgggggbaragacaatattagaaagagtgttc						2460
taggaggttatggaggacacggatgaggggtagaagggttagttaggtatttgagtgttgt						2520
ctggcttatcctttcatactagtagtcgtggaattatttgggtagtttcttgttttgta						2580
tttgatctttgttattctattttctgtttcttgtacttcgattattgtattatatactt						2640
gtcgtagttattgttcctcggtagaagtgtcttagcatgcttccttttagtgttttatcat						2700
gccttctttatattcgcggttgctttgaaatgcttttacttttagccgaggggtctattagaa						2760
acaatctctctatctcgtaaggtaggggtaaagtccctcaccacactccacttggtgggatt						2820
acattgtgtttgttgttgttaaatcaattatgtatacataataagtggattttttacaaca						2880
caaatacatgggtcaagggtcaaagttctgaacacataaagggttcattatatgtccagggga						2940
tatgataaaaaattgtttctttgtgaaagttatataagatttggtatggcttttgctggaa						3000

Fig 8 continued.



10/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
acataataagttataatgctgagatagctactgaagtttgttttttctagccttttaaat						3060
gtaccaataatagattccgtatcgaacgagtagtgttttgattacctggcatgatgtttc						3120
tattttttacatttttttgggtgttgaaactgcaattgaaaatgttgatcctatgagacgg						3180
atagttgagaatgtgttctttgtatggaccttgagaagctcaaacgctactccaataatt						3240
tctatgaattcaaattcagtttatggctaccagtcagtcagaaattaggatatgtgtgca						3300
tatacttgttcaattatactgtaaaatttcttaagttctcaagatatccatgtaacctcg						3360
agaatttctttgacagGCTTCTAGAAATAAGATATGTTTTCTTCTCAACATAGTACTGG						3420
A S R N K I C F P S Q H S T G						
ACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGAGT						3480
L K F G S Q E R S W D I S S T P K S R V						
TAGAAAAGATGAAAGGgtatgtttgataatttatatggttgcatggatagtatataaata						3540
R K D E R						
gttggaacttctggactggtgctcatggcatatttgatctgtgcaccgtgtggagatg						3600
tcaaacatgtgttacttcggtccgccaatttataataaccttaacttgggaaagacagctc						3660
tttactcctgtgggcatttggtatttgaattacaatctttatgagcatgggtgttttcaca						3720
ttatcaacttctttcatgtggtatataacagtttttagctccgttaataacctttcttctt						3780
tttgatataaactaactgtggtgcattgcttgcbkkkATGAAGCACAGTTCAGCTATTTT						3840
M K H S S A I S						
CGCTGTTTTGACCGATGACGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGAC						3900
A V L T D D D N S T M A P L E E D V K T						
TGAAAATATTGGCCTCCTAAATTTGGATCCAACCTTGGAACTTATCTAGATCACTTCAG						3960
E N I G L L N L D P T L E P Y L D H F R						
ACACAGAATGAAGAGATATGTGGATCAGAAAATGCTCATTGAAAAATATGAGGGACCCCT						4020
H R M K R Y V D Q K M L I E K Y E G P L						
TGAGGAATTTGCTCAAGgtaacagccaaaagttgtgcttttaggcagtttgaccttatttt						4080
E E F A Q G						
ggaagatgaattgtttatacctactttgactttgcttagagaattttgcataccggggagt						4140
aagtagtggctccatttaggtggcacctggccatttttttgatcttttaaaaagctgttt						4200
gattgggtcttcaaaaaagtagacaaggtttttggagaagtgacacacccccggagtgtc						4260
agtggcaaagcaaagattttactaaggagattcaaaatataaaaaaagtatagacataa						4320
agaagctgaggggattcaacatgtactatacaagcatcaaatatagtcttaaagcaattt						4380
tgtagaaataaagaaagtcttccttctgttgcttcacaatttccttctattatcatgagt						4440
tactctttctgttcgaaatagcttccttaataattaaattcatgatacttttgttgagatt						4500

Fig 8 continued

11/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
tagcagttttttcttgtgtaaactgctctctttttttg	cagGTTATTTAA	ATTTGGATT				4560
CAACAGGGAAGATGGTTGCATAGTCTATCGTGAATGGGCTCCTGCTCCTCagtaggtcct			Y L K F G F			4620
N R E D G C I V Y R E W A P A A Q						
cgtctactacaaaatagtagtttccatcatcataacagattttcctattaaagcatgatg						4680
ttgcagcatcattggctttcttacatgttctaattgctattaaggttatgcttctaatta						4740
actcatccacaatgcagGGAAGCAGAAGTTATTGGCGATTTC	CAATGGATGGAACGGTCT					4800
	E A E V I G D F N G W N G S					
AACCACATGATGGAGAAGGACCAGTTTGGTGTGGAGTATTAGAATTCCTGATGTTGAC						4860
N H M M E K D Q F G V W S I R I P D V D						
AGTAAGCCAGTCATTCCACACAACCTCCAGAGTTAAGTTTCGTTTCAAACATGGTAATGGA						4920
S K P V I P H N S R V K F R F K H G N G						
GTGTGGGTAGATCGTATCCCTGCTTGGATAAAGTATGCCACTGCAGACGCCACAAAGTTT						4980
V W V D R I P A W I K Y A T A D A T K F						
GCAGCACCATATGAGGTGTCTACTGGGACCCACCCTTCAGAAAGgttttgttattca						5040
A A P Y D G V Y W D P P P S E R						
taccttgaagctgaattttgaacaccatcatcacaggcatttcgattcatgttcttacta						5100
gtcttgttatgtaagacattttgaaatgcaaaagttaaaataattgtgtctttactaatt						5160
tggacttgatcccatactctttcccttaacaaaatgagtcaattctataagtgttgaga						5220
acttactacttcagcaattaaacagGTACCACTTCAAATACCCCTCGCCCTCCCAAACCCC						5280
	Y H F K Y P R P P K P R					
GAGCCCCACGAATCTATGAAGCACATGTGGCATGAGCAGCTCTGAGCCACGTGTAAATT						5340
A P R I Y E A H V G M S S S E P R V N S						
CGTATCGTGAGTTTGCAGATGATGTTTTACCTCGGATTAAGGCAAATAACTATAACTG						5400
Y R E F A D D V L P R I K A N N Y N T V						
TCCAGTTGATGGCCATAATGGAACATTCTTACTATGGATCATTTCGATATCATGTTACAA						5460
Q L M A I M E H S Y Y G S F G Y H V T N						
ACTTTTTTGTGTGAGCAGTAGATATGGAAACCCGGAGGACCTAAAGTATCTGATAGATA						5520
F F A V S S R Y G N P E D L K Y L I D K						
AAGCACATAGCTTGGGTTTACAGGTTCTGGTGGATGTAGTTCACAGTCATGCAAGCAATA						5580
A H S L G L Q V L V D V V H S H A S N N						
ATGTCACTGATGGCCTCAATGGCTTTGATATTGGCCAAGGTTCTCAAGAATCCTACTTTC						5640
V T D G L N G F D I G Q G S Q E S Y F H						
ATGTGGAGAGCGAGGGTACCATAAGTTGTGGGATAGCAGGCTGTTCAACTATGCCAATT						5700
A G E R G Y H K L W D S R L F N Y A N W						
GGGAGGTTCTTCGTTTCTTTTCCAACCTGAGGTGGTGGCTAGAAGAGTATAACTTTG						5760
E V L R F L L S N L R W W L E E Y N F D						
ACGGATTTCGATTGATGGAATAACTTCTATGCTGTATGTTTCATCATGGAATCAATATGG						5820
G F R F D G I T S M L Y V H H G I N M G						
GATTTCAGGAACTATAATGAGTATTTACCGGAGGCTACAGATGTTGATGCTGTGGTCT						5880
F T G N Y N E Y F S E A T D V D A V V Y						
ATTTAATGTTGGCCAATAATCTGATTCACAAGATTTTCCCAGATGCAACTGTTATTGCCG						5940
L M L A N N L I H K I F P D A T V I A E						
AAGATGTTTCTGGTATGCCGGGCTTGGCCGGCCTGTTTCTGAGGGAGGAATTGGTTTTG						6000
D V S G M P G L G R P V S E G G I G F V						

Fig 8 continued

12/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
TTTACCGCCTGGCAATGGCAATCCCAGATAAGTGGATAGATTATTTAAAGAATAAGAATG						6060
Y R L A M A I P D K W I D Y L K N K N D						
ATGAAGATTGGTCCATGAAGGAAGTAACATCGAGTTTGACAAATAGGAGATATACAGAGA						6120
E D W S M K E V T S S L T N R R Y T E K						
AGTGTATAGCATATGCCGAGACCCATGATCAGgtatttttaaattttatttctacaactaaa						6180
C I A Y A E T H D Q						
taattctcagaacaattgttagatagaatccaaatatatacgtcctgaaagtataaaagt						6240
acttatttttcgccatgggccttcagaatattggtagccgctgaatatcatgataagttat						6300
ttatccagtgacatttttatgttcactcctattatgtctgctggatacagTCTATTGTTG						6360
GTGACAAGACCATTGCATTCTCTCTAATGGACAAAGAGATGTATTCTGGCATGTCTTGCT					S I V G	6420
D K T I A F L L M D K E M Y S G M S C L						
TGACAGATGCTTCTCTGTTGTTGATCGAGGAATTGCGCTTCACAAGgtttgtctgtttc						6480
T D A S P V V D R G I A L H K						
tattgcattttaaggttcatataggttagccacggaaaatctcactctttgtgaggtaac						6540
caggggttctgatggattattcaattttctcgtttatcatttgtttattcttttcatgcat						6600
tgtgttttctttttcaatatccctcttatttggaggttaatttttctcatctattcactttt						6660
agcttctaaccacagATGATCCATTTTTTCACAATGGCCTTGGGAGGAGAGGGGTACCTC						6720
M I H F F T M A L G G E G Y L						
AATTTTCATGGGTAACGAGgtatgtcttacatcttttagatattttgtgataattacaatta						6780
N F M G N E						
gtttggcttacttgaacaagattcattcctcaaaatgacctgaactgttgaacatcaaag						6840
gggttgaaacatagaggaaaacaacatgatgaatgtttccattgtctagggatttctatt						6900
atgttgctgagaacaaatgtcatcttaaaaaaacattgtttactttttttagtataga						6960
agattactgtatagagtttgcaagtgtgtctgttttggagtaattgtgaaatgtttgatg						7020
aacttgtacagTTTGGCCATCCTGAGTGGATTGACTTCCCTAGAGAGGGCAATAATTGGA						7080
F G H P E W I D F P R E G N N W S						
GTTATGACAAATGTAGACGCCAGTGAACCTCGCGGATAGCGAACACTTGAGATACAAGg						7140
Y D K C R R Q W N L A D S E H L R Y K						
ttcaagtattttgaatcgagcttgttaataaatctagtaatttttagattgcttacttg						7200
gaagtctacttgggttctggggatgatagctcatttcattctgttctacttattttccaac						7260
cgaatttctgatttttgtttcgagatccaagtattagattcatttacacttattaccgcc						7320
tcatttctaccactaaggccttgatgagcagcttaagttgattctttgaagctatagttt						7380
caggctaccaatccacagcctgctatatttgttggatacttaccttttctttacaatgaa						7440
gtgataactaattgaaatggtctaaatctgatattctatatttctccgtctttcctccccct						7500

Fig 8 continued

13/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
catgatgaaatgcagTTTATGAATGCATTTGATAGAGCTATGAATTCGCTCGATGAAAAG	F M N A F D R A M N S L D E K					7560
TTCTCATTCTCGCATCAGGAAAACAGATAGTAAGCAGCATGGATGATGATAATAAGgta						7620
F S F L A S G K Q I V S S M D D D N K						
aatcatcctaaagtgtgaaagtgttgggtttatgaagtgtcttaattctatccaaggacaa						7680
gtagaaacctttttaccttccatttcttgatgatggatttcatatttttaatccaatag						7740
ctgggtcaaattcggtaatagctgtactgttagttactttcactttgcagGTTGTTGTGTT	V V V F					7800
TGAACGTGGTGACCTGGTATTTGTATTCAACTTCCACCCAAAGAACACATACGAAGGgta						7860
E R G D L V F V F N F H P K N T Y E G						
tatatgttttactttatccatgaaattattgctctgcttggttttaattgtactgaacaagt						7920
tttatggagaagtaactgaaacaaatcattttcacattgtctaatttaactcttttttct						7980
gatcctcgcatgacgaaaacagGTATAAAGTTGGATGTGACTTGCCAGGGAAGTACAGAG	Y K V G C D L P G K Y R V					8040
TTGCACTGGACAGTGATGCTTGGGAATTTGGTGGCCATGGAAGagtaaggatttgcttga	A L D S D A W E F G G H G R					8100
ataacttttgataataagataacagatgtagggtacagttctctcaccaaaaagaactgt						8160
aattgtctcatccatcttttagttgtataagatatccgactgtctgagttcggaagtgttt						8220
gagcctcctgcccctccccctgcgttggttagctaatcaaaaaggagaaaactgtttatt						8280
gatgatctttgtcttcatgctgacatacaatctgttctcatgacagACTGGTCATGATGT	T G H D V					8340
TGACCATTTCACATCACCAGAAGGAATACCTGGAGTTCAGAAACAAATTTCAATGGTGC	D H F T S P E G I P G V P E T N F N G R					8400
TCCAAATTCCTTCAAAGTGCTGTCTCTCGCGAACATGTGTGgtacagttcttgcctgtg	P N S F K V L S P A R T C V					8460
tgacctccctttttattgtggttttgttcatagttatttgaatgcatagaagttaacta						8520
ttgattaccgccacaatcgccagtttaagtcctctgaactactaatttgaaaggttaggaat						8580
agccgtaataaggtctacttttggcatcttactgttataaaaacaaaaggatgccaaaaaa						8640
attcttctctatcctctttttccctaaaccagtgcatgtagcttgcacctgcataaactt						8700
aggtaaatgatcaaaaatgaagttgatgggaacttaaaaccgccctgaagtaaagctagg						8760
aatagtcataataatgtccaccttttggtgtctgcgctaacatcaacaacaacatacctcgt						8820
gtagtcccacaaagtggtttcagggggagggttagagtgtatgcaaaacttactcctatct						8880
cagaggtagagaggattttttcaatagacccttggtcaagaaaaaaagtccaaaaagaa						8940
gtaacagaagtgaaagcaacatgtgtagctaaagcgacccaacttgtttgggactgaagt						9000

Fig 8 continued

14/17

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
agttgttgttgttgaacagtgcatgtagatgaacacatgtcagaaaatggacaacacag						9060
ttattttgtgcaagtcaaaaaaatgtactactatcttcttgtgcagctttatgtatagaa						9120
aagttaaataactaatgaattttgctagcagaaaaatagcttgagagaaaattttttata						9180
ttgaactaagctaactatattcatctttctttttgcttcttcttctctcttgtttgtgaag						9240
GCTTATTACAGAGTTGATGAACGCATGTCAGAACTGAAGATTACCAGACAGACATTGT						9300
A Y Y R V D E R M S E T E D Y Q T D I C						
AGTGAGCTACTACCAACAGCCAATATCGAGGAGAGTGACGAGAACTTAAAGATTTCGTTA						9360
S E L L P T A N I E E S D E K L K D S L						
TCTACAAATATCAGTAACATTGACGAACGCATGTCAGAACTGAAGTTTACCAGACAGAC						9420
S T N I S N I D E R M S E T E V Y Q T D						
ATTTCTAGTGAGCTACTACCAACAGCCAATATTGAGGAGAGTGACGAGAACTTAAAGAT						9480
I S S E L L P T A N I E E S D E K L K D						
TCGTTATCTACAAATATCAGTAACATTGATCAGACTGTTGTAGTTTCTGTTGAGGAGAGA						9540
S L S T N I S N I D Q T V V V S V E E R						
GACAAGGAAGTTAAAGATTCACCGTCTGTAAGCATCATTAGTGATGTTGTTCCAGCTGAA						9600
D K E L K D S P S V S I I S D V V P A E						
TGGGATGATTGAGATGCAAACGCTCTGGGGTGAGGACTAGTCAGATGATTGATCGACCCTT						9660
W D D S D A N V W G E D						
CTACCGATTGGTGATCGCTATCCTTGCTCTCTGAGAAATAGGTGAGGCGAAACAAAAAAT						9720
AATTTGCATGATAAAAAAGTCTGATTTTATGATCGCTATCCTCGCTCTCTGAGAAAGAAGC						9780
GAAACAAAGGCGACTCCTGGACTCGAATCTATAAGATAACAAAGGCGACTCCTGGGACTC						9840
GAATCTATAAGATAACAAAGGCAATTCCAAGACTTGAATCTATAAAAAATTTAGTTAAGA						9900
ATGATTAACGTCCGATCCTAATTCGAATCGAGGCATCTTACCACTCCATTGATAATTATA						9960
TAAGTCAATAAGTCATATAAWAGTATTAAAAACTAAATTGACTTGATCGGTCTATCAAAA						10020
ATMAGATMAAATTGTGTTTCATATGTAACATTTTTTGTGTGACAAATTAGCTTAATTACATC						10080
TTTCATGTGCAATAACAAAGAAATGATAGGAATTTAGAGATTCCAATTTTTTTGTGCCA						10140
CAATTAAGTTAATTACATCTTTTCATTTGCAATAACAAAGAAATGATAGGAATTTAGAGAT						10200
CCAGTGTCAATACACAACCTAGGCCAACATCGAAAGCATAACTGTAACTCATGCATGAA						10260
GAAATCAGTCGTAAAAATGAATAAATGCGACATAAAAAACAAATTGCATGTATCATTAATG						10320
TGACTTAACTACAAGTAAAAATAAATTTAACAAATGTAACCTAACTACAAGTAAAAATAA						10380
ATTGCTTCTATCATTAACAAACAAACAGAATTAAAAAGAAAAAACATACTAAATCTTAC						10440
CGTCATTGATAAAAAAATAACCAAATTCATAATGCAAGGAAACGAAACGCGTCTGA						10500

Fig 8 continued

15/17

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TCGGGTATCAACGATGAAATGGACCAGTTGGATCGACTGCCTGCACAACGTTAGGTATGC						10560
CAAAAAAAGAACACGATCCTTTGCACCCGTTTCGATGATTATCAGTATGTTCAAAAAA						10620
AACCTAAGTTCATCCCAGTGTAACAGCCCCAACATCTGCCCCAAGTAACAAAAACAA						10680
CCAATTTATCTTATCTTATCTGCCACAAAATAATCGGTTTCACACTATTCTCTTGTAT						10740
ACAAAATTGACAAGTAGGAAGGAGAGGAGTCATCCAAATAAACGGTGCACGTTCTTTGAG						10800
AAAAGTCTTATTTTCGTAAGATCCAATTTCAACAACTTTTCTTCAAGTCAAAATTCCT						10860
GATAGTGTATCTCCTCTCGACGACCTCTTGCAATTGAACGATCTCCGCTTATCATGAAAG						10920
TTGCTTGGATAACAAGTATTGCAAGGGGGGACAGTAGCTATTAAGTTAGTCGGCCCAAG						10980
GAAATGGAGGAGTGATAGTCTCGAATATTATTCACCTCTTTAGCATTACCCGGTCTGGCT						11040
TTAAGGAGTTACGTCTTTTACGCTCGCCAATTTCTTTTTTTAGAAATGGTTGGTGTCAAAA						11100
TCGCGAGTTGTGGAAGGTTCAAGTTACTCGATTTCGTGATTTTCAAGTATGAGTGGTGAGA						11160
GAGATTCGATATTTTCACGAGGTGTATTCGAGGTCTAGTAGAACGAAGGGTGTCACTAAT						11220
GAAAGTTTCAAGAGTTCATCATCATCTTCTTCTAGTAGATTTTCGCTTTCAAATGAGTAT						11280
GAAAATCTTCCTCTTTTCTATTGATTTTCTTCATTGTTTTCTTCATTGTTGTGGTTGTT						11340
ATTGAAAAGAAAGAAATTTATAACAGAAAAAGATGTCAAAAAAAGGTAAATGAAAGA						11400
GTATCATATACTTAAAGAGTTGCGTAGAGATAAGTCAAAAGAAACAGAATTATAGTAATT						11460
TCAGCTAAGTTAGAATTC						11478

Fig 8 continued

16/17

157

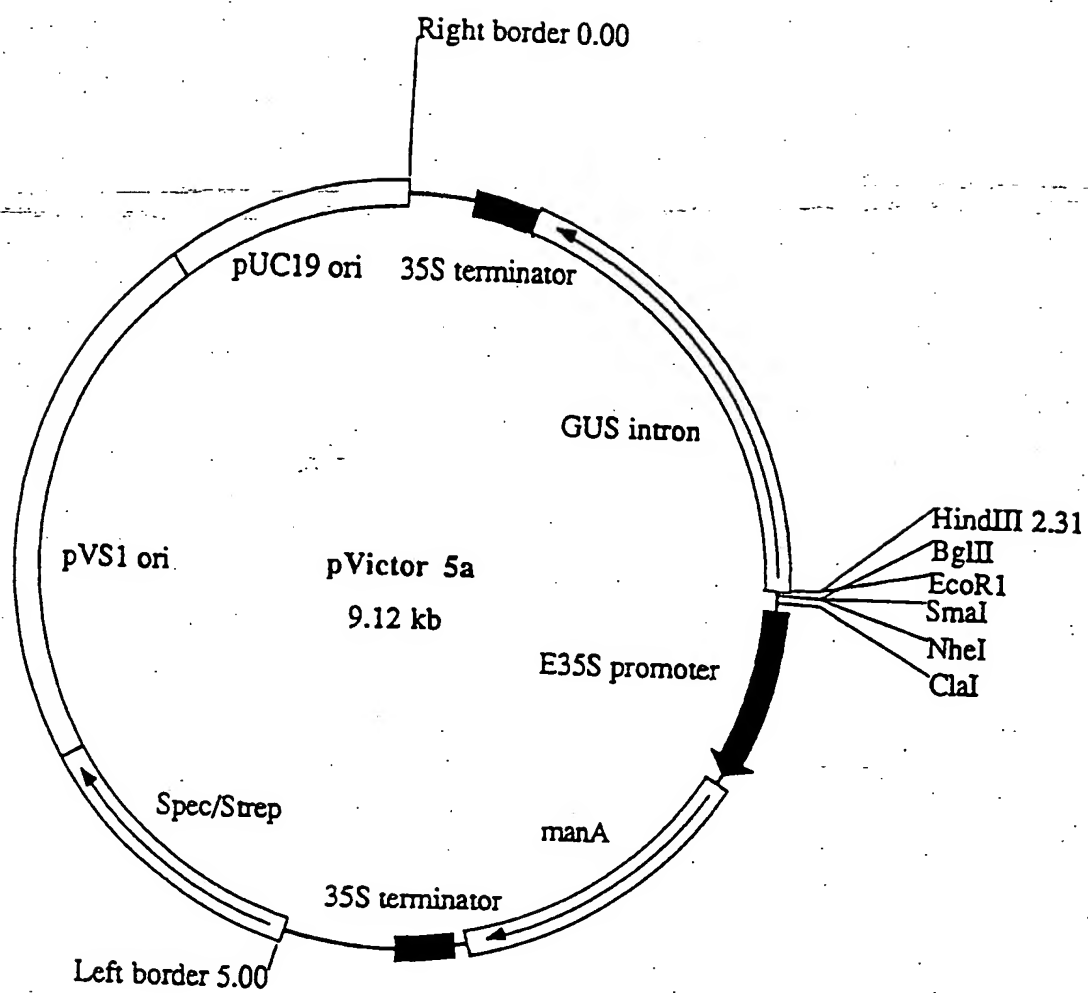


Fig 9

17/17

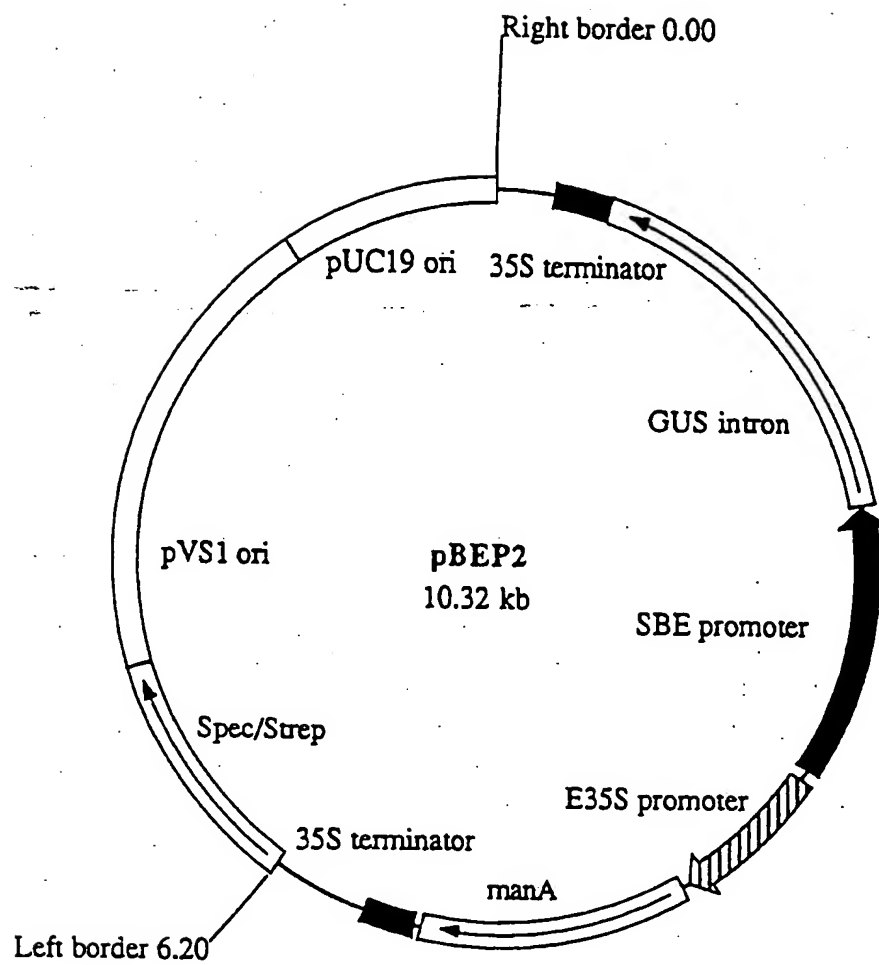



Fig 10



PCT/EP 96 / 03053

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 136is)

A. The indications made below relate to the microorganism referred to in the description on page <u>19</u> , lines <u>18 to 27</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution  The National Collections of Industrial and Marine Bacteria Limited (NCIMB)	
Address of depositary institution (including postal code and country)  23 St. Machar Drive Aberdeen Scotland AB2 1RY United Kingdom	
Date of deposit  13 July 1995	Accession Number  NCIMB 40754, NCIMB 40751, NCIMB 40752
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div>For receiving Office use only</div> <div><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div></div> <div>Authorized officer  R.L.R. Feather</div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer</div>

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

PCT/EP 96 / 03053

Danisco Biotechnology  
: gebugade 1  
P O Box 17  
DK-1001 Copenhagen K  
Denmark

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT  
issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Escherichia coli DH5 $\alpha$ -pBEA11	NCIMB 40754
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description	
<input checked="" type="checkbox"/> a proposed taxonomic designation	
(Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name: Address:	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 21 July 1995 <i>Teene Dando</i>
NCIMB Ltd 23 St Machar Drive Aberdeen Scotland UK AB2 1RY	

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

Danisco Biotechnology  
Langebrogade 1  
P O Box 17  
DK-1001 Copenhagen K  
Denmark

INTERNATIONAL FORM

PCT/EP96/03053

VIABILITY STATEMENT  
issued pursuant to Rule 10.2 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified on the following page

NAME AND ADDRESS OF THE PARTY  
TO WHOM THE VIABILITY STATEMENT  
IS ISSUED

<b>I. DEPOSITOR</b>	<b>II. IDENTIFICATION OF THE MICROORGANISM</b>
Name: As above  Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:  NCIMB 40754 Date of the deposit or of the transfer:  13 July 1995
<b>III. VIABILITY STATEMENT</b>	
The viability of the microorganism identified under II above was tested on 13 July 1995 <sup>1</sup> <input checked="" type="checkbox"/> <sup>3</sup> viable  <input type="checkbox"/> <sup>3</sup> no longer viable	

- <sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
- <sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.
- <sup>3</sup> Mark with a cross the applicable box.

7. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

## V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

**NCIMB Ltd**

Address:

23 St Machar Drive  
Aberdeen Scotland  
AB9 1PVSignature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

*Terence Dando*

21 July 1995

<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

DR A Buchter-Larsen  
Danisco Biotechnology  
Ingebrogade 1  
P O Box 17  
DK-1001 Copenhagen  
Denmark

INTERNATIONAL FORM

PCT/EP 96 / 03053

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT  
issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR:  Bacteriophage $\lambda$ EMBL3 SP6/T7 $\lambda$ SBE3.2	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:  NCIMB 40751
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:  <input type="checkbox"/> a scientific description  <input checked="" type="checkbox"/> a proposed taxonomic designation  (Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name: <b>NCIMB Ltd</b> <b>23 St Machar Drive</b> <b>Aberdeen Scotland</b> Address: <b>UK AB2 1FV</b>	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: <b>20 July 1995</b>

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

Dr A Buchter-Larsen  
Danisco Biotechnology  
Langebrogade 1  
P O Box 17  
DK-1001 Copenhagen K  
Denmark

## INTERNATIONAL FORM

VIABILITY STATEMENT  
issued pursuant to Rule 10.2 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified on the following page

NAME AND ADDRESS OF THE PARTY  
TO WHOM THE VIABILITY STATEMENT  
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above  Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40751  Date of the deposit or of the transfer:  13 July 1995
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 19 July 1995 <sup>2</sup> . On that date, the said microorganism was <div data-bbox="170 1291 341 1365"> <input checked="" type="checkbox"/> <sup>3</sup> viable         </div> <div data-bbox="170 1375 479 1449"> <input type="checkbox"/> <sup>3</sup> no longer viable         </div>	

<sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

<sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

<sup>3</sup> Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

## V. INTERNATIONAL DEPOSITARY AUTHORITY

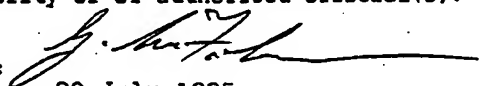
Name:

Address:

**NCIMB Ltd**  
23 St Machar Drive  
Aberdeen Scotland  
UK AB2 1RY

Signature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

  
20 July 1995

<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

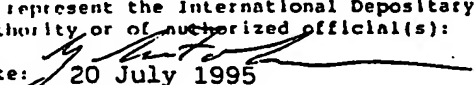
Dr A Buchter-Larsen  
Danisco Biotechnology  
Langebrogade 1  
Box 17  
DK-1001 Copenhagen  
Denmark

INTERNATIONAL FORM

**PCT/EP 96 / 03053**

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT  
issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page

NAME AND ADDRESS  
OF DEPOSITOR

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR: Bacteriophage $\lambda$ EMBL3 SP6/T7 $\lambda$ SBE3.4	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40752
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:  <input type="checkbox"/> a scientific description  <input checked="" type="checkbox"/> a proposed taxonomic designation  (Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name: <b>NCIMB Ltd</b> 23 St Machar Drive Aberdeen Scotland UK AB9 8QV	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 20 July 1995

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.



BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

Dr A Buchter-Larsen  
Danisco Biotechnology  
Langebrogade 1  
P O Box 17  
DK-1001 Copenhagen K  
Denmark

## INTERNATIONAL FORM

VIABILITY STATEMENT  
issued pursuant to Rule 10.2 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified on the following page

NAME AND ADDRESS OF THE PARTY  
TO WHOM THE VIABILITY STATEMENT  
IS ISSUED

<b>I. DEPOSITOR</b>	<b>II. IDENTIFICATION OF THE MICROORGANISM</b>
Name: AS above  Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40752  Date of the deposit or of the transfer:  13 July 1995
<b>III. VIABILITY STATEMENT</b>	
The viability of the microorganism identified under II above was tested on 19 July 1995 <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 45%;"> <sup>3</sup>  <input checked="" type="checkbox"/> viable         </div> <div style="width: 45%;"> <sup>2</sup> On that date, the said microorganism was         </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-start; margin-top: 10px;"> <div style="width: 45%;"> <sup>3</sup>  <input type="checkbox"/> no longer viable         </div> <div style="width: 45%;"></div> </div>	

- <sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
- <sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.
- <sup>3</sup> Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

## V. INTERNATIONAL DEPOSITARY AUTHORITY


Name:

**NCIMB Ltd**

Address:

23 St Machar Drive  
Aberdeen Scotland  
AB9 1RYSignature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

  
20 July 1995<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**